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SELECTIVE ADSORPTION BY SOILS

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From the standpoint of soil chemistry the absorption of material from the air and the soil solution by the soil is of first importance. The absorptive power of a soil enables it to retain the soluble salts necessary to plant life in spite of the leaching effect of rains and the movement of the soil solution toward the surface of the soil in dry weather, and thus to store up soluble material, either natural or applied in the form of a so-called fertilizer, for the future needs of crops.

The absorptive properties of soils have been under investigation in the Soil Laboratory for several years under the direction of Dr. Frank K. Cameron, and several publications [1] describing this work have appeared from time to time. The object of the work described in this paper was to obtain clearer insight into the mechanism of adsorption phenomena, particularly selective adsorption, and the characteristic effects of one solute upon the adsorption of another.

It is a well-known fact that either by leaching or by shaking a soil with a solution of potassium chlorid (or some neutral salt) the amount of potassium present will be diminished, and a certain amount of the bases of the soil (Ca, Mg, etc.) will be found in the resulting solution, while the amount of the chlorin will remain practically unchanged. Also, the resulting solution is slightly but distinctly acid to our common indicators.

On treating kaolin with solutions of magnesium and sodium chlorids Kohler [2] found the resulting solutions to be slightly but distinctly acid

[1] Cameron, F. K., and Bell, J. M. The mineral constituents of the soil solution. U. S. Dept. Agr., Bur. Soils, Bul. 30, 1905.

Cameron, F. K., and Patten, H. E. The distribution of solute between water and soil. Jour. of Phys. Chem., v. 11, p. 581–593, 1907.

Patten, H. E. Some surface factors affecting distribution. Trans. Amer. Electrochem. Soc., v. 10, p. 67–74, 1906.

Patten, H. E., and Gallagher, F. E. Absorption of vapors and gases by soils. U. S. Dept. Agr., Bur. Soils, Bul. 51, 1908.

Patten, H. E., and Waggaman, W. H. Absorption by soils. U. S. Dept. Agr., Bur. Soils, Bul. 52, 1908.

Schreiner, Oswald, and Failyer, G. H. The absorption of phosphates and potassium by soils. U. S. Dept. Agr., Bur. Soils, Bul. 32, 1906.

[2] Kohler, Ernst. Adsorptionsprozesse als Faktoren der Lagerstättenbildung und Lithogenesis. Ztschr. Prakt. Geol., Jahrg. 11, p. 49–59, 1903.

to litmus and attributed this to the fact that a selective concentration of the dissolved substance—an adsorption of the base—had taken place.

E. C. Sullivan[1] repeated these experiments and obtained the same result, accounting for it by an exchange of the sodium and magnesium of these salts in part for the iron and aluminium of the kaolin, the salts of the latter undergoing extensive hydrolysis in dilute solution.

Similarly, the acidity of a salt solution after treating a soil [illegible] explained by some as a hydrolysis of aluminium and iron salts after replacement by the base of the salts and by others as a selective adsorption of the base of the salt.

It has been found by many experimenters that on quantitatively determining the replaced bases present in a salt solution after treating a soil, kaolin, various silicates, etc., with the solution the replaced bases are equivalent or very nearly equivalent, within the limits of experimental error, to the loss of the base of salt.

Van Bemmelen[2] treated 100 grams of soil with 200 c. c. portions of solutions containing 8 and 40 mg. equivalents of potassium chlorid. After filtration the solutions were analyzed, and it was found that an almost complete exchange of potassium for sodium, calcium, and magnesium had taken place. Chlorin was determined in one experiment and had not changed.

Sullivan[3] found that by treating kaolin and various other silicates with salt solutions a quantity of bases almost equivalent to the loss of the base from the salt was dissolved in each case.

Wiegner[4] found that on treating an artificial amorphous water-containing (hydrated) so-called double silicate with a neutral salt solution the cation of the neutral salt was taken in part from the solution, and in its place the cations of the silicate-gel in nearly equivalent amounts entered the solution. The anion of the neutral salt remained unchanged, provided secondary reaction did not take place.

From many similar investigations with the same general result—namely, that the bases dissolved are very nearly equivalent to the loss of the base of the salt in solution—it would seem and is concluded by many experimenters that an exchange of bases takes place in the soil according to the following reaction:

$$KCl \text{ (for example)} + X_n \text{ silicate}_m \rightleftharpoons X_nCl + K \text{ silicate}_m$$

From the standpoint of fertilizer practice, however, on applying potassium chlorid to the soil it is very unlikely that the above reaction takes place and that the potassium is held in the soil as a relatively insoluble silicate and in a form highly unavailable for plants.

[1] Sullivan, E. C. The interaction between minerals and water solutions. U. S. Geol. Survey, Bul. 312, 1907.

[2] Bemmelen, J. M. van. Das Absorptionsvermögen der Ackererde. Landw. Vers. Stat., Bd. 21, p. 135-191, 1877.

[3] Sullivan, E. C. Op. cit.

[4] Wiegner, Georg. Zum Basenaustausch in der Ackererde. Jour. Landw., Bd. 60, p. 111-150, 197-272, 1912.

Certain inactive solid substances presenting large surfaces have the power of taking salts from solution—that is, what is known as absorbing or adsorbing them, a phenomenon most logically explained at present as a concentrating of the solute at the surface of the adsorbing material. Qualitatively, it is known that certain of these inactive solid substances not only have the power of adsorbing a neutral salt from its solution as a whole, but may adsorb one ion more than the other, or selectively adsorb. In so doing, a partial hydrolysis of otherwise practically unhydrolyzed salts is brought about, since the removal of one ion of the salt more or at a greater rate than the other takes an equivalent number of ions of opposite charge from the water and thus leaves an excess of either hydrogen or hydroxyl ions in the solution. That such is the case can be shown by the use of common indicators, after shaking solutions of neutral salts with or percolating them through certain of these inactive solid substances.

These cases are so numerous that only a few of the best known and more convincing ones will be here recalled.

A silver-nitrate solution shaken with animal charcoal and the supernatant liquid filtered and tested with methyl orange or litmus gives a distinct color of acid reaction.

A potassium chlorid or nitrate solution shaken with cane-sugar charcoal and the supernatant liquid filtered and tested with phenolphthalein gives a strong red color of alkaline reaction.

An interesting case of selective adsorption is to be found in our common indicator, Congo red, and absorbent cotton. If the base of a column of absorbent cotton is immersed in a solution of Congo red made very slightly acid, in a very few minutes the cotton immediately above the solution is colored blue (acid reaction), while above the blue color for about an inch in height is seen the red color of neutral or alkaline reaction; above the red the cotton is wet with water.

The soil possesses all the essential properties of these adsorbing materials; but that it has the power of selectively adsorbing to any appreciable extent has for a long time been a question of dispute. The fact that a solution of a neutral salt after contact with a soil is as a rule distinctly acid to indicators supports this hypothesis.

If a soil in contact with a solution of potassium chlorid adsorbs potassium ions at a much greater rate or in greater proportion than chlorin ions, thereby (since an equivalent number of hydroxyl ions are also removed with the potassium ions) causing a partial hydrolysis of the solution ($KCl + HOH = (KOH)$ adsorbed $+ HCl$), then free hydrochloric acid will be left in the solution.

It is not unreasonable to assume that the uncombined acid might dissolve an almost equivalent amount of bases from the soil particles. On this assumption, by using a solution of a salt of potassium with a weaker acid than hydrochloric, there should be a greater adsorption of potassium ions,

since the salt is more easily hydrolyzed than potassium chlorid, less surface energy being required to obtain potassium ions from solution, while the quantity of anions adsorbed will depend upon the specific properties of the anion employed. Also, if the anion of the salt is that of a weaker acid than hydrochloric and is not adsorbed to a much greater extent than chlorin ions, a smaller amount of bases should be dissolved from the soil and a correspondingly greater acidity of the solution should result. Again, if a reaction is interposed so that the free acid will be used up before it has a chance to react with the soil particles—i. e., by adding a small amount of sodium hydroxid, yet enough to neutralize the acid theoretically set free—little or no dissolved bases of the soil should be found in the resulting solution.

On the assumption that certain ingredients of the soil adsorb in part the base of a neutral salt in solution and that the free acid resulting from the hydrolysis caused by this adsorption reacts with certain of the soil particles and dissolves an almost equivalent amount of bases of the soil, the following experimental work is based.

SERIES No. 1

In series No. 1, 500-gram portions of a Durham sandy loam were introduced into a number of bottles of 2-liter capacity. To the first was added 2,000 c. c. of a solution containing 7.65 grams of potassium chlorid per liter; to the second 2,000 c. c. of a solution containing potassium acetate equivalent to 7.47 grams of potassium chlorid per liter; to the third 2,000 c. c. of water. The bottles were shaken frequently at room temperature for two days. The soil was allowed to settle until the supernatant liquid was apparently clear. Portions of the supernatant liquid were then pipetted off, filtered, and analyzed.

The supernatant liquid from soil shaken with pure distilled water showed no appreciable presence of material dissolved from the soil, while the analyses of the supernatant liquids from soil shaken with the above solutions showed soil material present. The potassium-chlorid equivalents of the various constituents determined by these analyses are given in Table I.

TABLE I.—*Adsorption by Durham sandy loam of potassium from solutions of potassium salts.*

[Results stated in grams of potassium chlorid per 100 c. c. equivalent to constituents determined by analyses.]

Constituents by analysis.	From KCl solution.	From CH_3COOK solution.	Constituents by analysis.	From KCl solution.	From CH_3COOK solution.
	Grams.	*Grams.*		*Grams.*	*Grams.*
K before contact.....	0.7650	0.7470	Mg after contact.....	0.0157	0.0167
K after contact.....	.6950	.6560	Na after contact.....	.....	.....
Al after contact.....	.0107	.0015	Free acid after contact.	.0112	.0492
Ca after contact.....	.0353	.0314	Anions after contact...	.7647	.7450

In the foregoing experiments the determination of the free acid is unreliable, considering the fact that no indicator could be used for titrating which was sensitive enough and at the same time unaffected by carbon dioxid. The results can be considered only as approximations. Boiling to remove the carbon dioxid is impossible when potassium acetate is used, since it hydrolyzes on boiling, giving an alkaline reaction to indicators. Iron and titanium were determined in several cases and found to be present in negligible amounts in the precipitated alumina. The amount of chlorin present in the solution was found to be practically unchanged.

From the data obtained when the potassium chlorid is used, the amount of potassium chlorid equivalent to loss of potassium (0.7650−0.6950= 0.0700 grams per 100 c. c.) during contact is greater than the amount of potassium chlorid equivalent to the bases dissolved from the soil (0.0107+ 0.0353+0.0157=0.0617 grams per 100 c. c.) by an amount (0.0700− 0.0617=0.0083 grams per 100 c. c.) about equal to the amount of potassium chlorid equivalent to the estimated free acid (0.0112 grams per 100 c. c.). When potassium acetate is used, the amount of potassium chlorid equivalent to the loss of potassium (0.7470−0.6560=0.0910 grams per 100 c. c.) during contact is again greater than the amount of potassium chlorid equivalent to the bases dissolved from the soil (0.0015+ 0.0314+0.0167=0.0496 grams per 100 c. c.) by an amount (0.0910− 0.0496=0.0414 grams per 100 c. c.) about equal to the amount of potassium chlorid equivalent to the estimated free acid (0.0402 grams per 100 c. c.).

When potassium acetate is used, the bases dissolved from the soil are 54.5 per cent $\left(\frac{0.0496}{0.0910}\times 100\right)$ of what they would be if a complete exchange of bases had taken place, while, when potassium chlorid is used, this percentage is 88.1 per cent $\left(\frac{0.0617}{0.0700}\times 100\right)$.

SERIES No. 2

In series No. 2, 250 grams of a Norfolk sandy loam were placed in a 2-liter bottle. To this was added 1,000 c. c. of a solution containing 18.38 grams of potassium chlorid and about 1 gram of sodium hydroxid per liter. The bottle was shaken frequently at room temperature for two days. The soil was allowed to settle until the supernatant liquid was apparently clear. Portions of the supernatant liquid were then pipetted off, filtered, and analyzed.

Soil shaken with pure water showed no appreciable presence of material dissolved from the soil in the supernatant liquid.

The above potassium-chlorid solution when shaken with soil showed a quantity of potassium chlorid equivalent to the loss of potassium of 0.1520 grams per 100 c. c. and no appreciable loss of chlorin. The

amount of bases of the soil (Ca, Mg, etc.) present in the resulting solution was found to be negligible. If, however, too great an excess of sodium hydroxid is present, the resulting solution is discolored, and iron in appreciable amounts is found in the solution.

It was found that the addition of a small amount of sodium hydroxid to a solution of potassium chlorid prevented the presence of dissolved bases when the solution is shaken up in contact with a soil, and yet a loss of potassium occurred of the same magnitude as when bases were found in the resulting solution, the amount of chlorin remaining practically unchanged.

Believing the assumption previously made to have been entirely justified by the foregoing experimental work, the hope of finding the effect of concentration, size of soil particles, and presence of other substances, with special regard to substances commonly used in fertilizer practice, on the selective adsorption by soils led to the following experimental work:

SERIES No. 3

In series No. 3, 35-gram portions of a Norfolk sandy loam collected near Laurinburg, N. C., and a Marshall silt loam collected near Edgerton, Mo., were placed in 200 c. c. bottles with solutions of potassium chlorid containing varying quantities of potassium chlorid and a small amount of sodium hydroxid per liter. The bottles were then rotated in a thermostat at room temperature for two days. The soil was allowed to settle until the supernatant liquid was apparently clear. Portions of the supernatant liquid were then pipetted off, filtered, and analyzed, the results of the analyses being given in Table II.

TABLE II.—*Effect of concentration on adsorption of potassium from solutions of potassium chlorid by Norfolk sandy loam and by Marshall silt loam.*

Norfolk sandy loam.				Marshall silt loam.			
Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Loss.		Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Loss.	
Before contact.	After contact.	Per 100 c. c. of solution.	Percentage.	Before contact.	After contact.	Per 100 c. c. of solution.	Percentage.
Grams.	*Grams.*	*Grams.*		*Grams.*	*Grams.*	*Grams.*	
25.8550	25.6750	0.1800	0.70	11.8400	11.3500	0.4900	4.14
14.7700	14.6500	.1200	.81	10.0450	9.5700	.4750	4.73
9.1250	8.9650	.1600	1.75	6.6950	6.2450	.4500	6.72
6.2580	6.1100	.1480	2.36	4.4860	4.0420	.4440	9.90
4.7400	4.5950	.1450	3.06	2.6700	2.2400	.4300	16.11
3.1120	2.9600	.1520	4.89	1.1640	.7700	.3940	33.81
1.8380	1.7010	.1370	7.45				
.6406	.5640	.0766	11.96				
.3064	.2650	.0414	13.51				
.1283	.0960	.0323	25.18				

From the data obtained in this experiment (see fig. 1) we find that from the zero concentration of potassium chlorid, where necessarily the adsorption of potassium is zero, the loss of potassium during contact increases regularly with the concentration to a certain point and then remains practically constant, the surface of the soil particles having apparently taken up the greater part of the potassium possible at this point. The point at which the adsorption of potassium becomes practically constant is much lower in the case where a sandy loam is used than when a silt loam is used. The percentage of potassium adsorbed increases asymptotically as the concentration of potassium chlorid

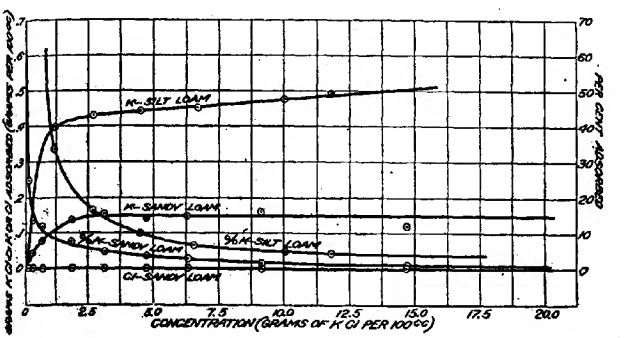

FIG. 1.—Curves showing the effect of concentration on the selective adsorption of potassium from solutions of potassium by Norfolk sandy loam and by Marshall silt loam.

decreases, and it may be concluded that the adsorption of potassium becomes practically complete at very low concentrations of potassium chlorid. Chlorin was determined in several cases and was found to have remained unchanged.

SERIES No. 4

In series No. 4, 35-gram portions of a subsoil of Cecil clay, a subsoil of Marshall silt loam, a subsoil of Norfolk sandy loam, a subsoil of Decatur clay loam, and a subsoil of Carrington loam were placed in 200 c. c. bottles with solutions of potassium chlorid of about the same concentration and treated as in series No. 3. The results are given in Table III.

TABLE III.—*Effect of amount of surface exposed on adsorption.*

Type of soil.[1]	Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Difference.
	Before contact.	After contact.	
	Grams.	*Grams.*	*Grams.*
Cecil clay	0.7350	0.4100	0.3250
Decatur clay loam	0.5550	0.3150	.2400
Marshall silt loam	0.6950	0.2450	.4500
Carrington loam	0.4300	0.2050	.2250
Norfolk sandy loam	0.2580	0.1100	.1480

[1] The soils in this table are arranged in order of the relative amount of surface exposed.

As was expected, since the removal or adsorption of potassium from a potassium-chlorid solution is undoubtedly a surface phenomenon, in general the smaller the soil particles the greater was the adsorption of potassium. Clay, however, in spite of the fact that the particles are smaller than those of the other types of soil, does not show a correspondingly greater adsorptive power, the surface of the clay particles being probably of a different nature. The classification of the different types of soil is based entirely on their mechanical analysis.[1]

SERIES No. 5

In series No. 5, 35-gram portions of Marshall silt loam (the same as that used in experiment III) were placed in 200 c. c. bottles with solutions containing varying amounts of potassium chlorid per liter. To some of the portions 10 grams of sodium nitrate were added, while to others 10 grams of monobasic calcium phosphate were added. These were treated as in experiment III. A solution containing 58.25 grams of potassium chlorid per liter in contact with calcium phosphate alone lost an amount of potassium during contact equivalent to 0.0500 gram of potassium chlorid per 100 c. c. The results of the analyses of the supernatant liquids are given in Table IV.

TABLE IV.—*Effect of the presence of other substances on adsorption.*

Experiment No.	Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Loss.	
	Before contact.	After contact.	Per 100 c. c. of solution.	Percentage.
	Grams.	*Grams.*	*Grams.*	
A.—With 10 grams of $NaNO_3$ present:				
I	11.1850	10.3750	0.8100	7.25
II	8.9950	8.2650	.7300	8.12
III	6.2400	5.6600	.5800	9.30
IV	4.4270	3.9470	.4800	10.83
V	2.0450	1.7140	.3305	16.15
VI	.8270	.5950	.2320	28.05
B.—With 10 grams of $CaH_4(PO_4)_2$ present:				
I	11.1100	10.5700	.5400	4.86
II	9.1300	8.6200	.5100	5.59
III	6.3400	5.8500	.4900	7.73
IV	4.5830	4.1200	.4630	10.10
V	1.9930	1.5480	.4405	22.10
VI	.9190	.5500	.3690	40.15
C.—With 5 grams of $NaNO_3$ present:				
I	6.3950	5.8200	.5750	9.00
D.—With 5 grams of $CaH_4(PO_4)_2$ present:				
I	6.3850	5.9000	.4850	7.60

Table IV and figure 2 show that the presence of sodium nitrate at concentrations of potassium chlorid below about 37.5 grams per liter

[1] Fletcher, C. C., and Bryan, H. Modification of the method of mechanical soil analysis. U. S. Dept. Agr., Bur. Soils, Bul. 84, 1912.

decreases the adsorption of potassium from a potassium-chlorid solution by a soil and increases it above this concentration. They also show that the presence of monobasic calcium phosphate does not alter the adsorption of potassium from a potassium-chlorid solution appreciably,

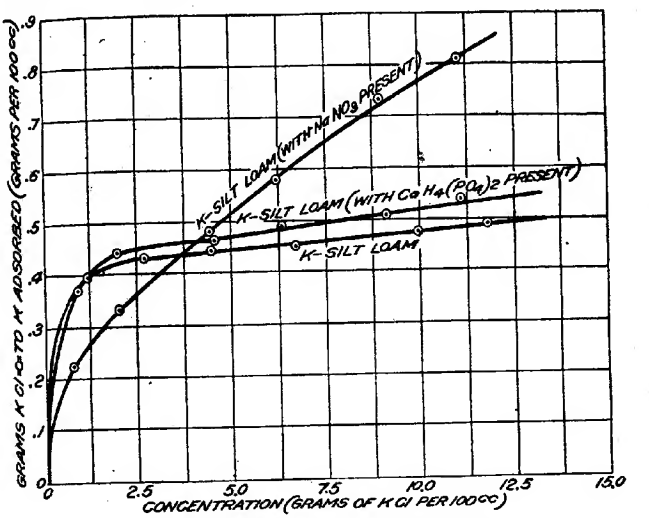

FIG. 2.—Curves showing the effect of the presence of sodium nitrate and calcium phosphate on the selective adsorption of potassium from solutions of potassium chlorid.

what change there is in the form of the curve being undoubtedly due to the removal of potassium by the calcium phosphate not in solution, either by a physical (adsorption) or a chemical reaction.

SUMMARY

Soils not only have the power of adsorbing dissolved salts from solutions but also of adsorbing one ion at a greater rate than the other, or selectively adsorbing, to a marked extent.

The presence of bases of the soil (Ca, Mg, etc.) in solution after shaking certain salt solutions with or percolating through a soil is probably not due to a direct chemical reaction of the salt in solution with the silicates of the soil, but to a reaction of free acid, resulting from a selective adsorption of the cation, with the mineral components of the soil.

The rate of adsorption of chlorin ions from solution by soils is much less than of potassium ions.

The selective adsorption of potassium from a potassium-chlorid solution by a soil increases in amount with the concentration up to a certain point and then remains practically constant.

The percentage of potassium adsorbed from a potassium-chlorid solution increases asymptotically as the concentration of potassium chlorid decreases and at very low concentrations adsorption is practically complete.

In general, the smaller the soil particles the greater the selective adsorption of potassium from a potassium-chlorid solution by the soil.

The presence of sodium nitrate decreases the adsorption of potassium from a solution of potassium chlorid by a soil up to a concentration of about 37.5 grams of potassium chlorid per liter and then increases it.

The presence of monobasic calcium phosphate does not change appreciably the adsorption of potassium from a potassium-chlorid solution by a soil.

Finally, if a mineral fertilizer be applied to a soil and exposed to the rain and thus dissolved and carried through the soil in solution, these substances will be adsorbed (an entirely physical phenomenon) either as a whole or selectively from the solution by the vast surface of the soil particles and will be held there by this same physical force until the plant or subsequent leaching removes it.

The presence of other mineral substances added to the soil may or may not increase or decrease the rate at which this adsorptive phenomenon takes place.

A BACTERIUM CAUSING A DISEASE OF SUGAR-BEET AND NASTURTIUM LEAVES

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INTRODUCTION

The bacterial disease described in this paper was first observed in the spring and summer of 1908 on nasturtium leaves growing near Richmond, Va., and on sugar-beet leaves collected from the Government plat at Garland, Utah. The disease on both hosts was of the leaf-spot type, but since the general appearance was not at all similar there was no thought at the time of a possible relationship between the causal organisms.[1] Investigations of the disease as it occurred on each host were at once begun, but not until the studies had progressed for nearly two years did it become evident that there was a striking similarity in regard to both cultural and morphological characteristics of the bacteria isolated from the two kinds of diseased leaves.

A comparative study of the bacteria followed, care being taken to use the same media placed under similar conditions. As a result of studies extending over four years, it has been found that in essential characteristics the bacterial organisms are so nearly identical that in the opinion of the writers the causal organism is one and the same bacterium. Any minor differences which occur may be attributed to individual adaptation due to host influence.

OCCURRENCE AND GENERAL APPEARANCE OF THE DISEASE ON THE TWO HOSTS

The material furnishing the basis of this study was received during the spring and summer of 1908. The diseased nasturtium leaves were sent in from Richmond, Va., to Dr. C. O. Townsend, then Pathologist in Charge of Sugar-Beet Investigations in the Bureau of Plant Industry. The diseased leaves had been gathered from young nasturtium plants growing in an open garden bed and when received were somewhat wilted and discolored, showing water-soaked and brownish-colored spots from 2 to 5 mm. in diameter. Upon microscopic examination the tissue within and surrounding these diseased spots was seen to be filled with great numbers of active bacteria.[2]

[1] Brown, Nellie A. A new bacterial disease of the sugar-beet leaf. Science, n. s., v. 29, no. 753, p. 915, 1909. Jamieson, Clara O. A new bacterial disease of nasturtium. Science, n. s., v. 29, no. 753, pp. 915-916, 1909.

[2] [Halsted, B. D.] Nasturtium blight. New Jersey Agr. Expt. Sta., 17th Ann. Rpt., [1895] 1896, p. 410, fig. 56, 1897.

The diseased sugar-beet leaves were collected by Dr. Townsend in Utah and California on inspection trips to the sugar-beet sections of the West and were sent to the laboratory in Washington for examination. Leaves similarly diseased were also received from Oregon during the summer of 1909, but, so far as known to the writers, the trouble has not been noticed up to the present time in any other beet-growing State.

The first leaves came from Utah and had dark-brown, often black, irregular spots and streaks from 3 mm. to 1.5 cm. in diameter. They occurred on the petiole, midrib, and larger veins. Occasionally the discoloration extended along the veins for some distance, and the tissue on either side was brown and dry; sometimes there were corklike protuberances at the central point of the spots. In badly diseased petioles the tissue had softened as though affected with a soft rot, but when only a few spots occurred there was no indication of softness.

Unlike the spot diseases due to Cercospora and Phyllosticta, this spotting did not spread through an entire beet field, but was generally limited to small areas.

The tissue embracing the dark spots was examined with the microscope as soon as the material was received and was found to be filled with very active bacteria; no fungous hyphæ were seen. Some of the leaves were placed in a moist chamber and carefully watched for several days, but there was no fungous mycelium in or around the spots.

ISOLATION OF THE ORGANISM FROM THE TWO HOSTS

The method of isolating the bacterial organism from the diseased sugar-beet and nasturtium leaves was by means of poured agar plates. Spots from the soundest leaves were used, the tissue being immersed in mercuric chlorid (1:1,000), washed in sterile water, and mashed in bouillon. The plate colonies were up in 24 hours. They were round, thin, smooth, glistening, whitish in reflected light, bluish in transmitted light, and 1 to 5 mm. in diameter. In three days the agar in the immediate neighborhood of the colonies had changed to a yellowish-green color. No other colonies appeared on the plates.

With young subcultures from these plate colonies needle-prick inoculations were made into sugar-beet and nasturtium plants, in order to prove that the right organism had been isolated in either case. The inoculations with the separate organisms from the two hosts are as follows:

INOCULATIONS WITH ORGANISM ISOLATED FROM SUGAR-BEET LEAF

Inoculations with the organism isolated from sugar-beet leaves into healthy sugar-beet leaves of plants growing in the greenhouse proved that the right organism had been isolated, for in three days there were black spots at all points of inoculation. The checks were free from

spots. Some of the inoculated leaves were taken to the laboratory, the black spots examined, and numerous bacteria found swarming in the cells. From these spots, produced by the first inoculations, the organism was reisolated in pure culture, and sugar-beet leaves in the greenhouse were inoculated repeatedly, the dark spotting and streaking of the leaves occurring in every case. Altogether, more than 100 sugar-beet leaves were inoculated. Although the infection took readily at the inoculated places, the disease was not observed to occur on any uninoculated beet plants except in two instances, when several beets of a neighboring row became affected. No slugs or worms were on the leaves, but thrips were abundant, and there were also a number of grasshoppers which had escaped capture; so possibly the infection was carried by one of these insects.

When the petioles, midrib, and large veins were inoculated by means of needle pricks, the infection took very rapidly, and the discoloration often ran along the course of the veins and veinlets. When the leaf blades were inoculated at the ends of tiny veins, there was only a darkened ring around the punctures. The infection took most rapidly on the petiole. (Pl. XVII, fig. 1.) In three days after needle-prick inoculations in young growing leaves the tissue was depressed, darkened, and often ruptured for a distance of 5 mm. around the puncture. Young beet leaves with blades about 8 cm. in length very readily succumbed to needle-prick inoculation in the blade as well as in the petiole and midrib. When material from a young culture less than 2 days old was inoculated into rapidly growing leaves, the spotting began to show in 24 hours. Old tissues were also found susceptible to the disease, but the infection did not take so rapidly. The sugar-beet root also was inoculated and the disease was found to take hold there slightly. (Pl. XVII, fig. 2.) There was no soft-rot condition, but cavities occurred in the roots where the inoculation pricks were made. These cavities penetrated into the interior of the beet and reached a depth of 2 cm. within two weeks after inoculation. Occasionally a cork-like condition of a dark color followed along the immediate line of the needle prick and no cavities were present. The discoloration, however, was not nearly so dark as in the leaf, nor was there as much tendency to spread as in the leaf.

So far as the writers know, this organism has not been found in the field attacking the beet root, and as none of the field beets with affected leaves had any root trouble, it is thought that the disease in the field is confined strictly to the leaf.

Spraying the organism on the leaves of beets did not produce the disease. Precautions were taken to prevent the bacteria from drying before they had time to get into the leaves. An infection cage was placed over beets growing in the open ground in the greenhouse, the

plants were watered well, and the leaves were sprayed with sterile water and left under the cage overnight, so that the stomata would open. The following day the growth from two-day-old agar cultures was shaken up well in sterile water and sprayed on the upper and lower surfaces of the leaves. The plants were watched carefully for two weeks, but no trace of the disease was ever seen. The experiment was repeated some months later with the same result.

Some cultures were sent to Garland, Utah, and Mr. H. B. Shaw, who had charge of the experiment station there during the season of 1909, inoculated the leaves of sugar beets growing in the open field. There, as well as in the greenhouse, the plants became infected very readily. Mr. Shaw sent some of the leaves to the sugar-plant laboratory at Washington. Upon examination swarms of bacteria were found in the blackened areas. Mr. Shaw also took portions of the diseased leaves, including the spots, and inoculated other leaves with them. Fifty per cent of the leaves treated in this way became spotted.

The most striking feature of this affection as it occurs in the greenhouse from inoculations is the black color of the spots and streaks, for they stand out prominently against the green of the leaves. These leaves never become soft, but bend over at the badly sunken spots, lose their turgidity, and finally die from drying out. If the petiole is inoculated, it frequently happens that the leaf blade will drop at a sharp angle from the infected area in less than two weeks.

INOCULATIONS WITH ORGANISM ISOLATED FROM NASTURTIUM LEAF

Inoculations with the organism isolated from nasturtium leaves were made into leaves of some rather old nasturtium plants growing in pots in the greenhouse. After several days small, watery-looking areas became visible, and the tissue within these areas became discolored and shriveled, resembling in all particulars the original spots from which the organism was obtained. A microscopic examination of the tissue within the diseased areas thus produced showed the cells to be filled with many active bacteria. Check plants having leaf surfaces pricked with a sterilized needle presented no indication of diseased spots. From the observation of inoculated plants it was noticed that the general appearance of the leaf spot changed considerably during the different stages of its development. Leaves of a healthy young nasturtium plant showed the effects of needle-prick inoculations within 48 hours, the tissue at first becoming slightly darker in the infected areas and presenting a water-soaked appearance. These spots gradually increased in size, becoming 4 to 6 mm. in diameter, while the tissue within became dry and brownish in color and often brittle enough to crack (Pl. XVIII). A dropping out of this diseased tissue frequently followed, and finally the whole leaf turned yellow and fell from the stem.

REISOLATION FROM INOCULATED TISSUE

Out of a small piece of tissue cut from one of the spots produced by inoculation a bacterial organism was isolated by means of agar plates, and by careful comparison with previous cultures was found to be similar in all respects to the organism obtained from the original diseased leaves. As soon as suitable cultures of this reisolated organism could be grown, inoculations were made into healthy young plants, and again the characteristic brown and shriveled spots were produced, with an abundance of active bacteria in the tissue. By these and other similar experiments it is proved beyond a doubt that the nasturtium leaf spot is caused by a bacterial organism. The manner in which the bacteria gain entrance to the tissue of the host has not been fully demonstrated, but from observations made during the investigation it seems probable that insect injuries, as well as mechanical wounds, open the way for the entering of the parasites.

CROSS-INOCULATIONS BETWEEN HOSTS

After proving that the right organism had been isolated from either host, inoculations into leaves of other plants were made, with the result that the sugar-beet organism proved very infectious to nasturtium, and likewise the nasturtium organism proved infectious to the sugar beet. But as the two investigators were working independently, each with one organism, this interesting fact had no particular significance at the time. Nasturtium leaves inoculated with the sugar-beet organism became spotted and watery-looking for some distance beyond the inoculation pricks, appearing in all respects similar to spots produced by inoculations with the nasturtium organism. Later, the watery-looking areas turned from a yellow to a brown color, and still later these tissues dried up and fell out (Pl. XIX, fig. 2). Some leaves drooped and died. The check leaves showed no discoloration; nor did any part of the tissue fall out, as in the inoculated leaves.

Three years afterward the same strain of the organism was inoculated into young nasturtium leaves at the same season of the year and under practically the same conditions as before, but there was a slight infection only, though young sugar-beet leaves inoculated with the same culture were badly infected.

Although inoculations with the nasturtium organism into sugar-beet leaves produced the disease, this strain of the organism was not so infectious as the sugar-beet strain on nasturtium. This difference in the behavior of the organisms in cross-inoculation was considered to be one of host influence.

OTHER PLANTS INOCULATED WITH THE ORGANISM FROM BOTH HOSTS

That this bacterial spot is not confined to sugar-beet and nasturtium leaves has been shown by a number of inoculations performed upon other plants growing in the greenhouse. Both strains of the organism were used. Diseased spots were produced with the bacteria upon leaves of pepper, lettuce, eggplant, and upon the leaves and pods of the bean plant. Inoculation experiments were also tried on potato, clover, and daisy plants, but without any definite infection, although there was slight discoloration on potato leaves.

The stems and leaves of the young pepper plants were readily infected through needle-prick inoculations. The spots were black, and the stems seemed more susceptible than the leaves.

Lettuce leaves growing in the greenhouse blackened readily after inoculation. One plant out of seven was entirely destroyed by the infection. One month later, when the temperature of the greenhouse was not so even throughout the day and night and the plants of the same lot had stopped growing rapidly and become toughened, the organism failed to produce infection.

The leaves of eggplant were inoculated, and brown spotting resulted at the punctured places; later, these areas dropped out of the leaves.

Of these various hosts the bean proved especially susceptible to the organism, inoculations taking effect almost as readily as upon the nasturtium and sugar-beet leaves. Bean plants inoculated with a young agar culture of both strains of the organism showed the characteristic brown spots on the leaves within three to five days. Ten days after inoculation some of the diseased leaves (Pl. XIX, fig. 1) were examined, and active bacteria were found in the cells. Three weeks after inoculation the bean leaves shriveled and died. Later, inoculations which were made upon the young pods of bean plants produced conspicuous, somewhat sunken, brownish spots in the tissue. (Pl. XIX, fig. 3.)

At the same time that the inoculation experiments were being carried on, cultural and morphological studies were made with both strains in the laboratory. From time to time notes and various tests were compared, and, as a result, the identity of the two strains was established. Such being the case, only one description will hereafter be given for the two strains, except where marked differences occur.

DESCRIPTION OF THE ORGANISM

MORPHOLOGICAL CHARACTERS

VEGETATIVE CELLS.—The organism is a medium-sized schizomycete of varying length when grown in different media. It is a short rod with rounded ends, occurring singly or in pairs (fig. 1); occasionally it occurs in long chains of two to many elements and again in long unsegmented

filaments (fig. 2, *a* and *b*). In stained tissue of the hosts the average measurement of a single rod is 1.2 by 0.6μ. The organism grown in a 3-day-old beef bouillon culture and stained in carbol fuchsin has an average size of 2.1 by 0.7μ. When stained with Loeffler's flagella stain, the average is 3.2 by 1.3μ.

FIG. 1.—*Bacterium aptatum* from a 2-day beef-bouillon culture stained with carbol fuchsin.

PROCESS OF CELL DIVISION.—Cell division takes place in the bacterium by simple, transverse fission. In order to study the process of fission, agar hanging blocks containing the organism were made in the following manner:

Thin beef-agar plates were poured and transfers from a bacterial culture streaked across the surface of the hardened agar. Agar blocks a few millimeters square were then cut out along the streak and transferred to clean cover slips. Care was taken to place the upper surface of the block next to the glass, after which the whole was turned over a Van Tieghem moist cell and kept at room temperature. At the end of 18 hours, by means of a microscopic examination of the agar block, with 2 mm. objective (oil immersion) and No. 6 ocular, bacteria were selected and their development through several generations was observed (fig. 3).

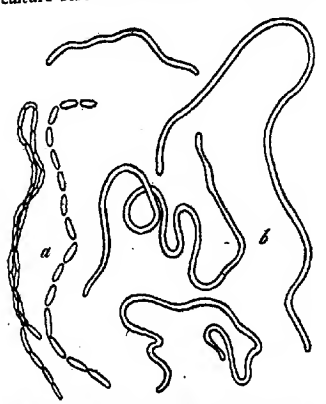

FIG. 2.—Filaments of *Bacterium aptatum* taken from the condensation water from a 2-day-old agar culture; stained with carbol fuchsin: *a*, Segmented; *b*, unsegmented.

FLAGELLA.—The organism is motile by means of polar flagella, varying from one to several at each pole. In general, the number is one to two, but occasionally three occur. The best results in staining flagella were obtained by the use of Loeffler's stain, with acid mordant correction. Five drops of sulphuric acid (the acid of such dilution that 1 c. c. is neutralized by the same amount of 1 per cent sodium hydroxid) were added to 15 c. c. of mordant. The flagella are threadlike, frequently wavy and somewhat tapering, often forming a loop or coil at the distal end, and are about twice as long as the body of the bacterium, actual measurement of 10 flagella giving an average of 4μ (fig. 4).

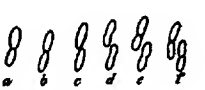

FIG. 3.—Process of cell division as seen in an 18-hour-old hanging drop culture of *Bacterium aptatum*. Time, *a* to *f*, 52 minutes.

QUESTION OF ENDOSPORES.—No spores have been demonstrated either by staining or testing with heat. Vacuolated forms were seen in cultures stained with spore stains. Several tests with heat were made, bouillon cultures 2 to 6 months old being treated as follows: Two were boiled three minutes and two were kept at 80° C. for 20 minutes; then transfers were made from both sets. These transfers were watched for nearly a month, but no trace of growth was seen. The transfers made before heating, as checks, showed a vigorous growth of the organism in two days. From these results it appears that spores are not formed by this bacterium, since, if present, they would have been carried over after the death of the vegetative cell, and growth would have been apparent in the new transfers. The fact that the bacterium is quite easily killed by atmospheric drying points to the same conclusion in regard to the absence of spores.

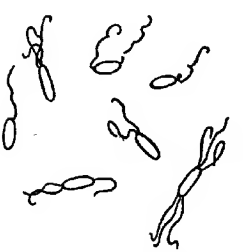

FIG. 4.—*Bacterium aptatum* showing flagella from a 2-day-old agar culture; stained with Loeffler's flagella stain.

INVOLUTION FORMS.—Involution forms are not common, but a few Y-shaped and cross-shaped forms were noticed in old cultures grown in media not favorable for the best development of the organism, such as beef bouillon containing 0.2 per cent of tartaric acid, or beef bouillon containing 0.1 per cent of oxalic acid. Some were found in ordinary media which had been placed under unusual conditions.

CAPSULES.—No capsules have been demonstrated. The organism is viscid after growing three days on agar and five to seven days in bouillon. Ribbert's and Richard Muir's capsule stains were used.

ZOOGLŒÆ.—Pseudozoogloeæ occur in +15 bouillon, Fermi's solution, bouillon containing salt, acids, and alkalies, and other liquid media in which the growth rises in a viscid swirl when the tube is shaken. When examined under the microscope, the viscid mass is found nearly always to be made up of short rods held in place by a network of gelatinous threads. Sometimes the mass is composed largely of the unsegmented filamentous bacteria (fig. 2, *b*).

BEHAVIOR TOWARD STAINS

The organism stains readily and uniformly in the ordinary basic aniline stains, such as methyl violet, gentian violet, saffranin, dahlia, fuchsin, and carbol fuchsin. It is not acid-fast and does not stain by Gram.

CULTURAL CHARACTERS

In general, the organism grows well upon many different kinds of artificial media, the most favorable for rapid and prolonged growth being +15 beef agar and bouillon, upon which it has been observed to live from 9 to 12 months.

AGAR PLATES.—At a temperature of 20° to 24° C. the colonies on peptonized beef agar (+15 on Fuller's scale) are up in 24 to 36 hours when plates are poured from a young bouillon culture. They are round, smooth, flat, glistening, 1 to 2 mm. in diameter, with entire edge, fish-scalelike markings, whitish in reflected light, bluish in transmitted light. In three days the colonies are 4 to 5 mm. in diameter on plates thinly sown, and the agar has changed to a faint yellowish green color. In 7 to 10 days the colonies are a deep-cream color.

AGAR STROKE.—There is a moderate growth along the stroke in 24 hours. It is whitish, flat, smooth, and glistening, spreading at base. In two days there is a heavy growth; in four days the agar has changed to a slight yellowish green, with the growth of a viscid consistency. In from five to seven days the bacterial growth covers nearly the entire surface of the agar, densely clouds the condensation water, and becomes slightly malodorous. The greatest growth of the organism occurs at the base of the stroke and in the condensation water. The margin of the stroke is often scalloped, with some edges of the scallop thinner than others. The growth on old cultures is a deep-cream color, the medium having become brown.

Tests made with the same organism, transferred at intervals for several years to artificial media, showed that the greenish color was not always produced in agar.

AGAR STAB.—Growth is slow in stab culture, only a slight trace occurring in two days. It is best at the surface; very little along the line of puncture. In four days the entire surface of the agar is covered with a whitish, smooth growth, and the agar at the top has changed to a faint yellowish green. Many crystals occur in the path of the needle. The agar is not liquefied or softened.

BEEF-BOUILLON CULTURES.—A slight clouding is noticeable in beef-bouillon (+15) cultures within 18 to 22 hours at room temperature (22° to 25° C.), increasing in density until a thick, viscid sediment forms in the bottom of the tube. When shaken, this sediment rises in a thick coherent, ropelike swirl. In bouillon cultures of three to five days' growth the solution becomes slightly greened, and a thin, whitish pellicle forms on the surface. This pellicle, which is composed of small masses of bacteria, is easily disturbed when shaken and falls in hundreds of tiny particles. In two weeks the medium has nearly cleared, a thick, whitish sediment has accumulated, and the solution is apple green in color, the fluorescence being most distinct toward the surface. In two months the medium has changed to a dark-amber color (Ridgway's "tawny"). Crystals may or may not occur.

NEUTRAL BEEF BOUILLON.—Growth occurs in 22 to 24 hours. There is a good growth in five days, and the medium has become a faint yellowish green color.

BOUILLON CONTAINING SODIUM CHLORID.—Growth occurred in neutral bouillon containing 5 per cent of sodium chlorid when tests were made with the organism soon after first isolating. Three years later these tests were repeated. Growth then took place in bouillon containing 3 and 3½ per cent of sodium chlorid, but there was no growth in bouillon to which 4 per cent of sodium chlorid was added.

BOUILLON OVER CHLOROFORM.—There is retardation of growth for two days; then the bouillon clouds and in nine days is colored a yellowish green tinge, as in the +15 bouillon without chloroform.

NITRATE-BOUILLON CULTURES.—In nitrate bouillon a thin clouding is produced within 24 hours, and in four days the solution is distinctly clouded, especially in its upper portion, where pseudozoogloœælike masses are visible. In eight days the thin pellicle which forms on the surface is easily shaken into many small particles. At this time a slight greenish cast appears in the solution. The same ropelike sediment described in beef bouillon was observed in a 9-week's-old culture of nitrate bouillon.

USCHINSKY'S SOLUTION.—In plain Uschinsky's solution and in the peptonized solution (1 per cent) strong clouding was produced in three to five days. In four days a thin pellicle composed of pseudozoogloœælike masses was observed. A greenish fluorescence became visible in five to eight days, and in three weeks the uniformly clouded solution had turned pale green (No. 328B, Code des Couleurs, Klincksieck et Valette).

FERMI'S SOLUTION.—There is a slight clouding in one day. In five days there is a thick tenacious pellicle, and the medium has changed to a decided pea-green color. A few fragments on the underside of the pellicle are suspended in the medium, and these occur in long gelatinous strings. On shaking the culture it is difficult to break up the pellicle and cause it to sink. In one month this pellicle is from 3 to 4 mm. thick.

COHN'S SOLUTION.—The organism does not grow in Cohn's solution.

STERILE MILK.—The milk is cleared slightly in two to four days, showing a gradual separation of whey from curd. This separation begins on the surface as a watery band and gradually extends downward, becoming complete in 12 days when kept at room temperature from 18 to 22° C. The medium is a yellowish cream color with a suggestion of green. There is a slight rim, but no pellicle. In one month the medium has become darker, and the green tinge has disappeared. It is translucent throughout. Compared with Ridgway's Color Chart, it is a clay color. After two months at room temperature the cultures are dried down 5 c. c., and are of a thick, creamy consistency. Transfers from these cultures showed that the organism was still alive.

LITMUS MILK.—In two days a blue ring appears at the surface of the liquid, extending down about 1 cm. In four days there are three rings

of graded shades of blue, while the lowest third of the liquid remains the color of the check tubes. Six to eight days later none of the original color of the liquid remains. Some tubes have four or five rings of color, the upper ring being the darkest blue. From 12 to 15 days after inoculating a brownish color appears at the bottom of the tube and extends upward, changing the entire liquid to a muddy blue in from three to six days. About four days later the medium begins to change to blue again and in seven days is entirely blue, approaching Ridgway's plum purple. Four different tests were made in which the color changes followed in this same manner. Room temperature, 18° to 22° C.

GELATIN PLATES.—Colonies of the bacterium which appear on gelatin (+10) plates within 48 hours are whitish, round, and glistening, with a smooth, flat surface having fishscalelike markings. Slight liquefaction began in two days at a temperature of 20° to 22° C., causing small clear areas around the colonies. In thickly sown plates liquefaction proceeded rapidly, becoming complete in three to five days. In plates thinly sown the liquefaction is only in cuplike areas about the colonies. When liquefied, the gelatin becomes a turbid, slightly greenish fluid.

GELATIN STAB CULTURES.—In gelatin (+10) stabs, growth was visible in two days on the surface about the stab, extending downward about 1 cm. (temperature 20° to 22° C.). Craterlike depressions with fluid contents were observed on the third day, increasing in size until a layer of fluid was formed. In 10 days this layer had become 1 cm. in depth. Liquefaction of the gelatin stab culture was complete in 30 days.

STEAMED POTATO CYLINDERS.—In three days growth on this medium is abundant, flat, smooth, cream white, and glistening. The potato changes to a gray-brown color in 3 days, and in 15 days is from two to four shades darker. The bacterial slime approaches Ridgway's wood brown. There is no diastasic action of the starch.

STARCH JELLY.—Growth is scant on starch jelly. In seven days the medium at the surface and about 3 mm. below the streak along which the growth of the organism has taken place has changed to a delicate green. The test for sugar with Fehling's solution was negative.

LOEFFLER'S BLOOD SERUM.—The growth is moderate and slow, scarcely a trace occurring in three days. The medium becomes gray and at the end of 32 days has liquefied a little. The stroke is filiform, flat, glistening, and smooth. The heaviest growth occurs in the condensation water.

LITMUS-LACTOSE AGAR.—Copious growth developed within two weeks in litmus-lactose agar cultures. The condensation water first clouded, after which growth began to show at the base of the stroke. In eight days there was growth along the entire stroke, with a spreading at the base and a pellicle formation in the condensation water. The medium was blued. At the end of nine weeks the growth was azure blue in color (No. 401, Code des Couleurs, Klincksieck et Valette).

GENTIAN-VIOLET AGAR.—Growth of the bacterium on gentian-violet agar was very slow, no growth being visible in 4 days and only a slight growth in 18 days. When examined four weeks after inoculation, however, a thin bluish growth was observed along the stroke and spreading from the base over the surface of the slant. The medium had paled, some of the violet color having been extracted by the bacterium in its growth.

OTHER CULTURAL FEATURES OF THE ORGANISM

NITRATES.—Nitrates are not reduced. Tests were made with nitrate bouillon cultures 5 and 10 days old in the following manner: 1 c. c. of a potato-starch solution was added to each culture, then 1 c. c. of a fresh potassium-iodid solution (1:250), after which 5 drops of dilute sulphuric acid (2:1) were added. There was no change of color in any of the 5 or 10 day old cultures.

INDOL.—No indol is present in cultures 1 to 10 days old. It is present, however, in cultures 11 to 25 days old. The tests were made as follows:

Transfers were made from a 2-day-old bouillon culture to Uschinsky's solution containing 2 per cent of peptone. These cultures grew at room temperature, 20° to 24° C., tests being made at the end of 1, 3, 5, 8, 10, 11, 12, 13, 15, and 25 days. Ten drops of concentrated sulphuric acid were added to each culture to be tested and after standing for five minutes, 1 c. c. of a 0.02 per cent solution of sodium nitrite was added. If no pink color was visible in the cultures five minutes after adding the nitrite, the tubes were heated to a temperature between 70° and 80° C. The rose color which indicates the presence of indol was not present in any of the tests up to the tenth day.[1] Indol was present in some of the 11-day cultures, but in the 15-day and 25-day cultures each one gave the definite rose-color reaction.

TEST FOR HYDROGEN SULPHID

No hydrogen sulphid is produced. Litmus-lactose agar slants were inoculated from a 2-day beef-agar culture. Small strips of filter paper previously moistened in a saturated solution of lead acetate were inserted in the tubes, being held in place by means of cotton plugs in such a manner as to prevent contact with the medium. In two days there was growth along the entire stroke, accompanied by a bluing of the agar, but without any discoloration of the filter paper. In six days the bacterial growth had become abundant, spreading at the base of the stroke and filling the condensation water. During a period of four weeks there was no evidence of hydrogen sulphid. The test was repeated with litmus-lactose agar, beef agar, and beef-bouillon cultures with the same result.

[1] In a few instances a faint pinkish color appeared on the tenth day in tests made with the nasturtium strain of the organism.

TEST FOR AMMONIA

The organism produces ammonia. Beef-bouillon cultures (2 to 8 weeks old) were tested with Nessler's solution. Strips of filter paper were moistened with the solution and suspended in the tubes to be tested. The cultures were then heated in a water bath. A brownish red color appeared on the filter paper and in the drops of distillate which collected on the sides of the tube. This coloration indicated the presence of ammonia in the cultures. A second test for ammonia was made by placing 25 c. c. of the Nessler's solution in large-sized tubes. Ordinary test tubes of beef bouillon inoculated with the bacterium were put into these larger tubes. The inner tubes were left open and the outer tubes closed with cotton plugs. After five days a brownish precipitate had formed in the Nessler's solution, forming a ring on the glass tubes at the surface of the liquid. Check tubes used in both tests did not show this precipitation.

TOLERATION OF ACIDS

Toleration of acids by the bacterium was tested in different percentages of tartaric, oxalic, and hydrochloric acid made up in beef bouillon. The organism was transferred from bouillon to acid cultures ranging from 0.1 per cent to 0.3 per cent solutions. Clouding occurred in 1 day in the tartaric acid in a 0.2 per cent solution, but there was no clouding in 10 days in a 0.3 per cent solution. In a 0.1 per cent solution oxalic acid there was slight clouding in 1 day, moderate clouding in 2 days, and strong clouding in 3 days, but no clouding in a 0.2 per cent solution. In the 0.1 per cent solution of hydrochloric acid, growth was slow in appearing; the solution became turbid in 1 to 2 weeks, and a greenish color was produced in the medium. No growth occurred in 0.125 per cent solution of hydrochloric acid during 10 days. A final test for acid toleration was made in beef bouillon containing hydrochloric and tartaric acids (titrating on Fuller's scale from +19 to +35). Results of this test showed heavy clouding in 5 days in +30 beef solution of both hydrochloric and tartaric acids, while no trace of clouding appeared in the +35 acid bouillons during 4 weeks.

TOLERATION OF SODIUM HYDROXID.—The toleration of sodium hydroxid by the bacterium is moderate. Transfers from a 7-day beef-bouillon culture clouded −15 beef bouillon in 1 to 2 days, −18 in 10 days, and occasionally a slight growth occurred in −20 after 2 weeks, but there was no clouding in −25 beef bouillon during a period of 4 weeks.

OPTIMUM REACTION FOR GROWTH IN BOUILLON.—The optimum reaction for growth in beef bouillon is between +15 and +30; the organism grows nearly as well at +25 as at +15, and the medium becomes fluorescent as in +15.

GAS FORMATION.—The organism is aerobic and does not form gas. Tests were made in fermentation tubes with water containing 2 per cent of Witte's peptone to which was added 1 per cent of each of the following carbon compounds: Glycerin, saccharose, mannite, maltose, dextrose, and lactose. (Levulose and galactose were used in addition with the strain of the organisms isolated from nasturtium.) No gas formed in any of the tubes. Because of differences between the two strains in regard to the clouding of solutions in the closed end of some of the fermentation tubes, the results of the tests are given separately.

With the organism isolated from sugar beet there was a heavy growth in the open arm of the tubes, but none in the closed ends. Dextrose and saccharose gave an acid test with litmus after the organism had been growing in the tubes 16 days. Glycerin, mannite, maltose, and lactose gave an alkaline test.

From inoculations with the organism isolated from nasturtium the following readings were made after 5, 10, and 28 days:

TABLE I.—*Readings from fermentation tubes inoculated with the nasturtium strain of the bacterium.*

Peptonized water with 1 per cent solution of—	After 5 days.	After 10 days.	After 28 days.
Lactose	Solution clouded in open end.	Clouded in open end and outer two-thirds of U tube; sharp line of demarcation; perfectly clear in closed end; no pellicle; litmus test, alkaline.	Clouded in open end and outer part of U tube; whitish precipitate; no growth in closed end; litmus test, alkaline.
Levulose	do	Clouded in open end and outer two-thirds of U tube; clear in closed end; no pellicle and no flocculence; litmus test, alkaline.	Clouded in open end and outer U tube; clear in closed end; litmus test, alkaline.
Maltose	do	Uniformly clouded in open end and outer two-thirds of U tube; sharp line of demarcation; no pellicle; clear in closed end; litmus test, alkaline.	Clouded in open end, with whitish precipitate; no growth in closed end; litmus test, alkaline.
Mannite	do	Clouded in open end and in U tube; no sharp line of demarcation; no pellicle; perfectly clear in closed end; litmus test, alkaline.	Clouded in open end and U tube; clear in closed end; no pellicle; whitish precipitate; litmus test, alkaline.
Glycerin	do	Uniformly clouded in open end and outer two-thirds of U tube; sharp line of demarcation; no pellicle; clear in closed end; litmus test, alkaline.	Clouded in open end, with whitish precipitate; clear in closed end; litmus test, alkaline.
Dextrose	do	Uniform clouding in open end and whole of U tube; no pellicle; clear in closed end; litmus test, acid.	Well clouded in open end, with numerous small particles in suspension; closed end clear, except a slight clouding in lower end; no pellicle; litmus test, distinctly acid.
Galactose	do	Clouded in open end and in U tube; no distinct line of demarcation; faint clouding in closed end; no pellicle; litmus test, distinctly acid.	Well clouded in open end, with many small particles in suspension; clouded in two-thirds of closed end; no pellicle; considerable precipitate; litmus test, distinctly acid.
Saccharose	do	Uniformly clouded in open end and in U tube; no sharp line of demarcation; no pellicle; clear in closed end; litmus test, feebly acid.	Thinly and uniformly clouded in open end and outer two-thirds of U tube; sharp line of demarcation; clear in closed end; no pellicle; whitish precipitate; litmus test, distinctly acid.

From Table I it may be seen that growth occurs in the open end of the fermentation tube in each of the nine solutions tried, while in the closed end there is slight clouding in dextrose and a distinct clouding in presence of galactose. In the test for alkaline and acid reactions neutral litmus paper was used. As a result of this test six of the sugar solutions showed an alkaline reaction and three (dextrose, galactose, and saccharose) showed a distinctly acid reaction. No gas formation was observed in the closed arm of any of the solutions during a period of 30 days.

TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. The test was made as follows:

Fresh transfers were made to beef bouillon from a 24-hour bouillon culture and placed in a Novy jar containing a solution of pyrogallic acid and sodium hydroxid (1 gram of pyrogallic acid to 10 c. c. of a 10 per cent solution of sodium hydroxid for each 100 c. c. of air space).

The control cultures were kept under normal conditions at room temperature.

The Novy jar was waxed and clamped tightly and connected on one side to a series of wash bottles containing pyrogallic acid and sodium hydroxid and on the other side to the exhaust. There were stopcocks to regulate the passing of the gasses through the jar. In the jar with the cultures was a fermentation tube which had its closed arm filled with water except for a bubble of air at the top. This bubble was noted as an indicator of pressure within the jar. As the oxygen was absorbed by the solution within the jar, air was allowed to pass in from the wash bottles until the bubble in the fermentation tube indicated the normal pressure. The exhaust was used to draw off the gases from the jar.

The operation was repeated several times during a period of three hours, after which the Novy jar was sealed and set aside. The atmosphere in the jar was then practically one of nitrogen. At the end of six days the cultures were taken from the jar and examined. There was no trace of clouding in the bouillon. The controls, however, showed heavy growth; in fact they were heavily clouded within two days.

This test was made a second time, the Novy jar being set up in the same way and the bouillon transfers made from a 24-hour culture as before. This time the jar was sealed for two weeks. When it was opened no growth could be detected in any of the bouillon cultures, while the controls showed the usual heavy growth after two days. The cultures which had been kept in the Novy jar were clouded heavily five days after they were removed.

TEMPERATURE RELATIONS

THERMAL DEATH POINT.—The thermal death point is 47.5° to 48° C. when transfers are made from a 24-hour bouillon culture and the inoculated tubes are kept at that temperature in the water bath for 10 minutes,

readings being taken at half-minute intervals during that time. Many tests were made, using for transfers +15 bouillon cultures 18 hours to 6 days old. When 3 to 6 day old cultures were used and kept in the water bath for 10 minutes at 51°, the organism was not killed; nor was it killed at 53° C. for the same length of time.

MAXIMUM TEMPERATURE.—The maximum temperature for the organism isolated from sugar beet is 35° C., while the maximum temperature for the organism from nasturtium is 33° to 34° C.

MINIMUM TEMPERATURE.—The minimum temperature is between 0° and −1° C. When kept at a temperature of −2° to −5° C. for five days by means of an ice and salt mixture, the organism remains alive and begins to grow after being restored to room temperature. A good growth of the organism occurs in both agar and bouillon at 11.5° C. A fair growth occurs in bouillon at 8° C.

OPTIMUM TEMPERATURE.—The optimum temperature is 27° to 28° C.

RELATION TO LIGHT

The organism is not especially sensitive to sunlight. Thinly sown agar poured plates were exposed in bright sunlight at midday in mid-winter on bags of crushed ice out of doors, half of each plate being covered with black paper to serve as a check. The test with the organism isolated from sugar beet was as follows:

Fifty minutes exposure did not kill the organism, for colonies appeared on the exposed side of these plates in two days, but no colonies appeared on those plates exposed 60 minutes. Three different tests were made. The organism isolated from nasturtium proved more resistant to sunlight, since a few scattered colonies appeared on the agar plates even after an exposure of 80 minutes.

RELATION TO MOISTURE

The beet organism is killed very readily by drying, even at a moderate or low temperature. When drops of a 1-day-old, well-clouded bouillon culture are placed on sterile cover glasses and kept in the dark at a temperature of 21° to 25° C. from four to five hours, growth occurs in bouillon tubes into which these covers are dropped. When kept six hours, all the organisms are dead. With 3 to 6 day old cultures treated in the same way the organism was able to withstand drying from one to three days.

VITALITY IN CULTURE MEDIA

This organism lives from 10 to 12 months in liquid media, such as beef bouillon, sterile milk, and Fermi's solution, when kept at temperatures varying from 11° to 20° C. Bouillon cultures may die in four months and less when the plugs in the tubes are loose and such rapid evaporation occurs that the culture dries down. This usually takes place in the

summer at room temperature, 24° to 30° C. Beef-agar cultures live from 4 to 10 months, depending upon the temperature under which they are grown. Those cultures which die in from four to five months are grown at temperatures of 24° to 30° C.

LOSS OF VIRULENCE

No loss of virulence was noticed in the organism isolated from nasturtium until April, 1910 (two years after the first isolation), when inoculations were made into nasturtium and bean plants growing in the greenhouse. Five days after inoculation no apparent discoloration of the tissue could be observed. This result was unusual, since in all past inoculations the diseased spots had been readily produced. After repeated inoculations had been made from cultures of the bacterium grown in beef bouillon upon agar slants and potato cylinders it became evident that the organism, which had been growing on artificial media for two years, had lost its virulence.

In the case of the organism isolated from the sugar-beet leaf, no loss of virulence was noticed until about three years after obtaining the organism, and up to that time practically every needle-prick inoculation into sugar-beet leaves proved infectious. After three years the percentage of positive results from inoculations fell off considerably, as only the youngest leaves, growing under the proper conditions of moisture and temperature, became diseased. Efforts were made in the summer of 1911 to obtain a new strain of the organism from the field, but they were unsuccessful. Later, string-bean agar was tried and proved to be a rejuvenator of the organism isolated from both hosts. After growing on this medium, the organism was almost as infectious to sugar-beet leaves and nasturtium leaves as when it was first isolated. This virulence, however, was not permanent, for in the course of a year it became much reduced.

BACTERIA IN CELL TISSUE

Diseased tissue produced in both hosts by inoculation was fixed, embedded in paraffin, sectioned, and stained in carbol fuchsin. Microscopic examinations of these sections showed the presence of bacteria in large quantities within the cells of the diseased tissue (fig. 5). In sections cut through the central portion of the diseased spots the walls appeared ruptured or collapsed. The cells at the margins of these ruptured places show that the bacteria are in the cells, although most of the bacteria were seen in the broken-down tissues adjacent to the sound cells.

NATURAL INFECTION AND CONTROL

Since practically all of the work has been done under laboratory and greenhouse conditions, there has been no opportunity to investigate the complete life cycle of this organism or to follow out the natural means of

infection in the field. This being the case, no practical methods of control have been undertaken, but in order to determine if possible something in regard to the way in which the organism gains an entrance into the tissue of its hosts, young plants were placed in infection cages in the greenhouse and the foliage sprayed with a bacterial solution until it was thoroughly wet. This solution was prepared from 5-day-old cultures of the organism. Check plants were placed in a control-infection cage and sprayed with distilled water. Examination was made at intervals of several days, but no diseased spots appeared on either the nasturtium or sugar-beet leaves during a period of 20 days. The result of the experiment suggests that infection takes place only in bruised or wounded tissue, due to insects or to mechanical injury.

FIG. 5.—Camera-lucida drawing of a portion of a cross section of sugar-beet leaf inoculated with *Bacterium aptatum*. The cells containing bacteria were next to many collapsed cells.

TECHNICAL DESCRIPTION OF THE ORGANISM

Bacterium aptatum, n. sp.

According to the numerical designations adopted by the Society of American Bacteriologists, the group number of *Bacterium aptatum* is 211.2322133.

Form, a short motile rod with rounded ends; flagella, bipolar; involution forms rare; no spores or capsules observed; pseudozoogloeæ occur; aerobic; smooth whitish colonies on agar plate with fishscalelike markings; clouds beef bouillon in 18 to 24 hours; produces alkaline reaction in litmus milk, with a gradual separation of whey from curd; liquefies gelatin; produces ammonia; no reduction of nitrates; fluorescence greenish; no diastasic action on potato starch; grows in Uschinsky's and Fermi's solutions; indol produced after 10 days; optimum temperature 27° to 28° C.; maximum 34° to 35° C.; minimum — 1° C.; thermal death point 47.5° to 48° C.; vitality 4 to 10 months in beef agar, 10 to 12 months in bouillon, depending on temperature; good growth on litmus-lactose agar; growth much retarded on gentian-violet agar; stains readily with basic anilin dyes; not acid-fast; not stained by Gram; tolerates acids; oxalic, 0.1 per cent; tartaric, 0.2 per cent; hydrochloric, 0.1 per cent; tolerates sodium hydroxid in beef bouillon, — 18 Fuller's scale; no growth in Cohn's solution; killed readily by drying; not very sensitive to sunlight; retains its virulence 2 to 3 years; pathogenic to nasturtium, sugar-beet, and several other plants.

COMPARISON OF PSEUDOMONAS TENUIS WITH BACTERIUM APTATUM

While the work on *Bacterium aptatum* was being prepared for publication, Bulletin No. 167 of the Vermont Experiment Station was received,[1] part 3 of which contains a description of green fluorescent bacteria

[1] Edson, H. A., Jones, C. H., and Carpenter, C. W. Micro-organisms of maple sap. Vermont Agr. Exp. Sta. Bul. 167, p. 321–610, 14 fig., 16 pl., 1912.

occurring in maple sap. Results of a comparative study of seven representative strains of the green fluorescent sap bacteria and six known fluorescent species are given, and the group numbers of these organisms determined. Since one of these numbers, that of *Pseudomonas tenuis*,[1] is identical with the group number of *Bacterium aptatum*, it was found necessary to make cultural comparisons. A culture of *Pseudomonas tenuis* was obtained by Dr. Erwin F. Smith from Mr. C. E. A. Winslow, American Museum of Natural History, who stated that he had received it from Mr. Edson.

Table II shows the results of comparative tests made with *Pseudomonas tenuis* and *Bacterium aptatum*.

TABLE II.—*Comparison of the characteristics of Pseudomonas tenuis and Bacterium aptatum.*

Media, etc.	Pseudomonas tenuis.	Bacterium aptatum.
1. Beef bouillon	Rapid clouding; green fluorescence; distinct pellicle.	Clouding with green fluorescence; distinct pellicle.
2. Beef-agar stroke	Smooth, thin, whitish growth; medium greened.	Smooth, thin, whitish growth; medium greened.
3. Uschinsky's solution	Strong clouding with fluorescence; pellicle formed.	Strong clouding with fluorescence; pellicle formed.
4. Nitrate reduction	None.	None.
5. Indol test	No indol in 10-day cultures, but present in 16-day cultures.	Indol present in 10 to 12 days.
6. Hydrogen - sulphid test.	Hydrogen sulphid produced.	No hydrogen sulphid.
7. Gelatin plates	A trace of liquefaction in 3 weeks on thickly sown plates.	Liquefaction begins on second day and is complete in 5 days in thickly sown plates.
8. Gelatin stabs	No liquefaction in 3 weeks.	Liquefaction begins in 2 to 3 days.
9. Sterilized milk	Gradual thickening in 6 weeks without clearing.	Clearing begins in 2 to 3 days and is completed in 2 weeks.
10. Litmus milk	Alkalin reaction; color uniform throughout during 7 weeks.	Alkalin reaction; banded appearance resulting in clearing and a uniformly blue color in 3 to 4 weeks.
11. Ammonia test	Ammonia produced.	Ammonia produced.
12. Pathogenicity	Nonpathogenic to sugar-beet and nasturtium leaves.	Pathogenic to sugar-beet and nasturtium leaves.

From results given in Table II it is evident that *Pseudomonas tenuis* and *Bacterium aptatum*, although closely related in the green fluorescent group of bacteria, do not belong to the same species. Similarity of growth occurs and was especially noticed in beef bouillon, on beef agar, and in Uschinsky's solution. *Pseudomonas tenuis*, however, clouds bouillon and Uschinsky's solution more quickly than *Bacterium aptatum*. Both organisms produce indol and ammonia. Neither reduces nitrates. *Pseudomonas tenuis* has a strong putrefactive odor not present in cultures of *Bacterium aptatum*. *Pseudomonas tenuis* produces hydrogen sulphid, while *Bacterium aptatum* does not. In sterilized-milk cultures, *Bacterium aptatum* gradually separates whey from curd, and in litmus milk this process is accompanied by changes of color, giving a distinctly banded appearance during the first week's growth. Neither the separation of

[1] Zimmermann, O. E. R. Die Bakterien unserer Trink- und Nutzwasser . . . Reihe 1, Chemnitz, 1890. 106 p. Also in 11. Bericht, Naturwissenschaftliche Gesellschaft, Chemnitz, 1887, 1889, p. 53–154, 1890.

Thumm, Karl. Beiträge zur Biologie der fluorescierenden Bakterien. Arb. Bakt. Inst. Karlsruhe, Bd. 1, Heft 3; p. 291–377. [1895.]

whey from curd nor the color changes were apparent in cultures of *Pseudomonas tenuis* during a period of seven weeks. One of the most important cultural differences between these two organisms appeared on gelatin plates. *Bacterium aptatum* is a rapid liquefier, while *Pseudomonas tenuis* showed only a trace of liquefaction in three weeks, this slight liquefaction occurring only on thickly sown plates and not at all in stab cultures. The essential difference, however, between *Bacterium aptatum* and *Pseudomonas tenuis* is not so much a cultural as a physiological one. This is shown in the ability of *Bacterium aptatum* to produce diseased spots on sugar-beet, nasturtium, and bean leaves, while *Pseudomonas tenuis* is nonpathogenic to these hosts.

COMPARISON OF BACTERIUM PHASEOLI WITH BACTERIUM APTATUM

When it was observed that *Bacterium aptatum*[1] produced diseased spots so readily on leaves of the bean plants, the question at once suggested itself as to the relation between this organism and *Bacterium phaseoli*, the cause of the well-known bacterial blight of bean, as described and worked out by Dr. Erwin F. Smith.[2] The cultural characteristics of *Bacterium aptatum* were, therefore, compared with those of *Bacterium phaseoli*. As a result of this comparison it is evident that the two organisms are entirely different.

Some of the characteristic differences between the two organisms are shown in Table III.

TABLE III.—*Comparison of the cultural characteristics of Bacterium aptatum and Bacterium phaseoli.*

Media, etc.	Bacterium aptatum.	Bacterium phaseoli.
Beef agar (plate)	Whitish colonies, slightly bluish in diffused light; medium greened.	Yellow colonies, smooth, wet-shining; thin, distinct margins.
Agar slant	Whitish, smooth, faintly blue in transmitted light; medium greened.	Smooth, translucent, yellow; slimy consistency; growth without retardation.
Potato slant	Cream white to wood-brown; viscid; medium browned; no diastasic action.	Copious yellow slimy growth, medium grayed; diastasic action powerful.
Litmus milk	Alkalin reaction; slow clearing during seven weeks.	Slow alkalinity and separation of casein from whey.
Thermal death point	47.5° to 48° C.	49.5° C.
Flagella	Bipolar; one to several	Polar; one.
Pathogenic to—	Nasturtium, sugar beet, bean, and other plants.	Bean and lupine.
Resistance to dry air	Few hours to several days.	27 days.
Resistance to sunlight	80+ minutes	30 to 45 minutes.
Color in mass	Whitish	Yellow.

[1] This comparison was made with *Bacterium aptatum* isolated from nasturtium.

[2] Smith, E. F. Description of Bacillus phaseoli n. sp., with some remarks on related species. Proc. Amer. Assoc. Adv. Sci., 46th meeting, 1897, p. 288–290, 1898.

—— The cultural characters of Pseudomonas hyacinthi, Ps. campestris, Ps. phaseoli, and Ps. stewarti—four one-flagellate yellow bacteria parasitic on plants. U. S. Dept. of Agr., Div. Veg. Physiol. and Path., Bul. 28, 153 p., illus., 1901.

—— Bacteria in Relation to Plant Diseases. v. 2, Washington, D. C., 1911, p. 62. (Carnegie Inst. Washington, Pub. 27, v. 2.)

COMPARISON OF BACTERIUM XANTHOCHLORUM WITH BACTERIUM APTATUM

While investigations with *Bacterium aptatum* were in progress, attention was called to the recent work of Dr. Julius Schuster upon a bacterial decay of the potato tuber caused by *Bacterium xanthochlorum*.[1] From Dr. Schuster's description it was observed that in morphological and certain cultural characters this potato bacterium resembled quite closely *Bacterium aptatum*. Since both belong to the green fluorescent group of bacteria, it seemed worth while to take up a comparative study of the two organisms. Fortunately a culture of Dr. Schuster's *Bacterium xanthochlorum* was at hand, having been brought to our laboratory by Dr. H. W. Wollenweber in November, 1911. Accordingly a series of cultural tests was begun at once and continued for a period of about three months.[2] As a result of these tests it is evident that *Bacterium aptatum* and *Bacterium xanthochlorum* are not identical, although their appearance is quite similar upon some kinds of culture media. Table IV gives a partial record of the results obtained and will be sufficient to show the differences.

TABLE IV.—*Comparison of the cultural characteristics of Bacterium aptatum and Bacterium xanthochlorum.*

Media.	Bacterium aptatum.	Bacterium xanthochlorum.
+15 beef-agar plates	Growth less rapid than Bacterium xanthochlorum; fishscalelike markings on surface colonies pronounced.	Growth more rapid and appearance of colonies more compact than those of Bacterium aptatum.
+15 beef-agar stroke	Growth less rapid than Bacterium xanthochlorum and greenish fluorescence not so marked.	Growth rapid and fluorescence marked.
+15 beef-agar stab	Growth whitish to drab color in center of nail head.	Growth pinkish colored in center of nail head.
+10 gelatin plates	Growth slower than Bacterium xanthochlorum and liquefaction does not begin so early; medium only slightly greened.	Growth and liquefaction rapid; medium distinctly greened.
+15 beef bouillon	Thin pellicle of pseudozoogloeælike masses; sediment a ropelike viscid swirl; fluorescense appears slowly.	Growth rapid; pellicle membranous and falling entire; green fluorescence striking.
Potato cylinders	Appearance similar to Bacterium xanthochlorum.	Growth gradual; at first creamy white, later brownish; starch not broken down.
Nitrate bouillon	Less rapid growth than Bacterium xanthochlorum; pellicle easily breaking into small particles; fluorescence weak.	Growth rapid; pellicle membranous and breaking into fragments; fluorescence much greater than Bacterium aptatum.
Sterile milk	Slow separation of whey from curd; no distinct fluorescence; pellicle of floating islands.	Separation of whey from curd more rapid than in Bacterium aptatum; pellicle more distinct; greenish fluorescence marked.
Litmus milk	Color of whey blue with whitish rim formed around tube above solution; pellicle not complete.	Color of whey grayish; rim above solution pink to purplish; pellicle distinct.
Uschinsky's solution	Clouding less dense than Bacterium xanthochlorum; fluorescence moderate; pellicle composed of pseudozoogloeæ-like masses.	Clouding dense; pure green fluorescence; membranous pellicle.
Litmus-lactose agar	Growth less rapid than Bacterium xanthochlorum; blue in color; medium blued; precipitate lead colored.	Growth rapid and dense; color of growth, greenish blue; medium blued; precipitate brownish.

[1] Schuster, Julius. Zur Kenntnis der Bakterienfäule der Kartoffel. Arb. K. Biol. Anst. Land- u. Forstw., Bd. 8, Heft 4, p. 452–492, 13 fig., pl. 5, 1912.

[2] The bacterium isolated from nasturtium leaves was used in these tests.

TABLE IV.—*Comparison of the cultural characteristics of Bacterium aptatum and Bacterium xanthochlorum*—Continued.

Media.	Bacterium aptatum.	Bacterium xanthochlorum.
Gentian-violet agar.....	Growth of streak much retarded; no growth during first 4 days; after 18 days, moderate growth; medium paled.	No retardation; copious growth in two days; blue in color; medium greened.
Fermentation tubes.....	Acid reaction in peptonized saccharose, in peptonized galactose, and in peptonized dextrose solutions.	Alkaline reaction in peptonized saccharose solution; acid reaction in peptonized galactose and in peptonized dextrose solutions.

SUMMARY

1. The leaf-spot diseases of sugar beet and nasturtium described in this paper are due to a bacterial organism.

2. The two diseases occurred during the same summer. The causal organism was isolated in pure cultures from both hosts and proved infectious to sugar-beet and nasturtium leaves interchangeably.

3. It is proved from cultural, morphological, and inoculation tests that the organisms causing these leaf-spot diseases on both hosts are identical.

4. The organism is also infectious to bean leaves and pods, lettuce, pepper, and eggplant.

5. It probably enters the plant through wounds or by means of insect injuries and may be spread by insects.

6. The organism is a bacterium belonging to the green fluorescent group. It is proved to be different from *Bacterium xanthochlorum*, which is pathogenic to potato, and from *Pseudomonas tenuis*, which has been given the same group number.

7. It is also different from *Bacterium phaseoli*, although both organisms produce spotting of bean leaves and pods.

8. The name *Bacterium aptatum*, n. sp., is suggested.

DESCRIPTION OF PLATES

PLATE XVII. Fig. 1.—Sugar-beet leaves inoculated with *Bacterium aptatum*. Photographed eight days after inoculation.

Fig. 2.—Sugar-beet root inoculated with *Bacterium aptatum*. Photographed two weeks after inoculation.

XVIII (colored). Nasturtium leaves showing bacterial leaf spots 10 days after inoculation with *Bacterium aptatum*. (May, 1909.)

XIX. Fig. 1.—Bean leaves inoculated with *Bacterium aptatum* from leaf-spot of sugar beet.

Fig. 2.—Nasturtium leaves inoculated with *Bacterium aptatum* from leaf-spot of sugar beet.

Fig. 3.—Bean pods inoculated with *Bacterium aptatum* from leaf-spot of sugar beet.

(Inoculated Nov. 12, 1908; photographed Nov. 25, 1908.)


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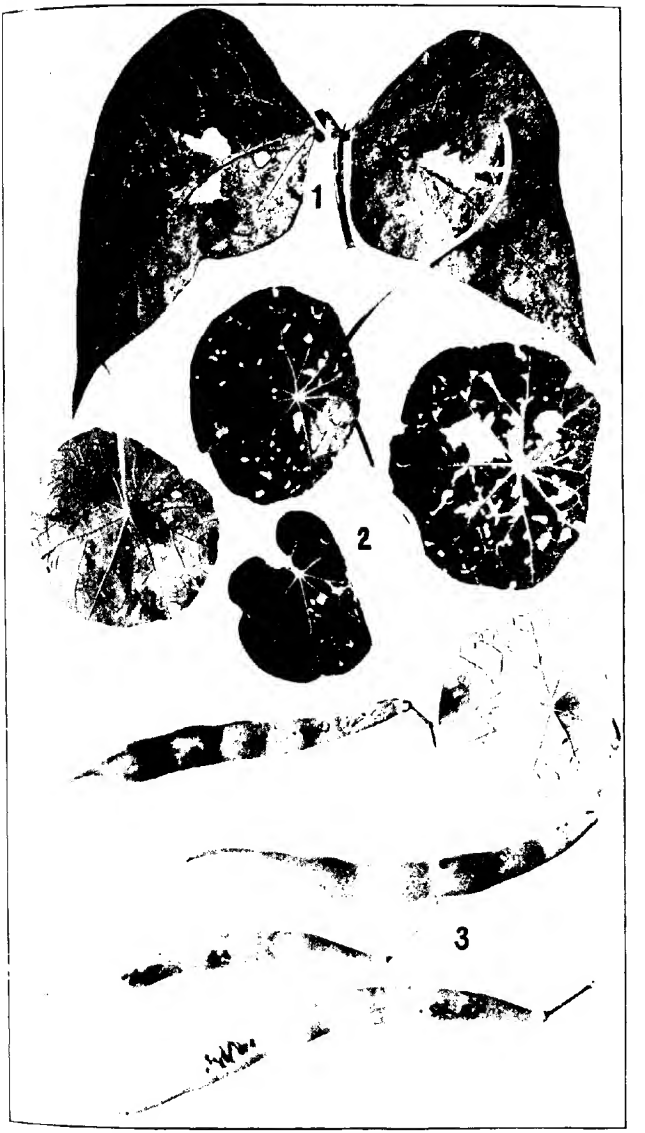
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THE CALLIEPHIALTES PARASITE OF THE CODLING MOTH

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INTRODUCTION

The notes and observations on which the present paper is based were obtained at Vienna, Va., under the direction of Prof. A. L. Quaintance, in Charge of Deciduous Fruit Insect Investigations, Bureau of Entomology, the writer having been assigned to work on the parasites of deciduous fruit insects at the Vienna laboratory in the spring of 1911.

So much has been published concerning the Calliephialtes parasite of the codling moth, under the names *Calliephialtes messor* Grav. and *Ephialtes carbonarius* Christ, since its introduction into California that it seemed advisable to begin the work on the project with a study of this species and its liberation on a large scale. The specimens with which the start was made were obtained from two lots of parasitized codling-moth larvæ secured in 1911 from the California State Insectary. The propagation from the first lot was unsuccessful, only three diminutive males being reared. The second lot was received in the late summer. These were reared to maturity, 15 females and a larger number of males being secured. After these had mated they were given access to codling-moth larvæ that had been compelled to spin their cocoons in strips of strawboard. The parasites oviposited very readily in the codling-moth cocoons. The progeny of these individuals did not emerge until the following spring. A large majority were lost in an attempt to force them through to early maturity in a greenhouse, where, in spite of daily soakings with water, the pupæ dried up. A few females forced to maturity in this way deposited eggs, but only males came from them. However, 21 females and 52 males were reared later from unforced material, and it was with these that the real start in the work was made in the spring of 1912.

During the season of 1912 several hundred individuals of both sexes were reared under observation from egg to maturity. The results of these observations are recorded in the following pages.

While the major part of the work was performed by the writer, it was greatly facilitated by the work of Mr. J. D. Luckett, half of whose time during the period from June 15 to September 15, 1912, was spent in assisting in this work.

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IDENTITY AND INTRODUCTION OF THE SPECIES

When the California State Horticultural Commission began its work of introducing this parasite into California in an attempt to control the codling moth, specimens were submitted to Dr. William H. Ashmead for determination. Dr. Ashmead determined them as the *Calliephialtes messor* of Gravenhorst, a species inadequately described from a single female specimen from Russia. Up to the time of the introduction into California, *C. messor* had been mentioned in literature only once since its description. This was by Taschenberg, who in 1863 recorded it as having been reared as a parasite of (*Tinea*) *Galleria mellonella*, the wax moth.

When the writer took up the work on the species, specimens reared from the codling moth in material sent to the Bureau of Entomology from Sachsen, Germany, were submitted to Mr. H. L. Viereck, who determined them as *Calliephialtes comstockii* Cress.; a species described from the United States. Later, specimens reared by the writer as progeny of the specimens received from California were sent to Dr. A. Roman, of the Stockholm Museum. Dr. Roman reported that the museum had no specimens of *C. messor*, but that those sent were identical with a specimen determined for the museum by Dr. Ashmead as *C. pusio* Walsh, another species described from America. The specimen in the Stockholm Museum bears only the label "Long I." Dr. Ashmead therefore evidently determined the same thing under two specific names, one European and the other American.

INTRODUCTION INTO CALIFORNIA

Late in 1904 Mr. George Compere, acting as an agent of the State Horticultural Commission of California, found this species attacking the codling moth in Spain. Living specimens were sent by him to California, where they were propagated and their progeny released in infested orchards. At this time the species was supposed to be *Ephialtes carbonarius* Christ, and references to it under that name have appeared in literature, but specimens from California were determined by Dr. William H. Ashmead as *messor* Grav. and the species placed in his genus Calliephialtes. That it is not *Calliephialtes carbonarius* is firmly established by the well-known habit of that species of attacking wood-boring insects.

In view of the uncertainty as to the specific identity of the parasite, the writer has avoided the use of any specific name in the present paper.

INTRODUCTION INTO SOUTH AFRICA

From California specimens of the species were sent to the Cape of Good Hope in 1907, where they were propagated and released by the Government Entomologist, Prof. C. P. Lounsbury. Reports of the results of this introduction indicate that it is of doubtful success.

DESCRIPTION OF THE SPECIES

GENERAL DESCRIPTION

The adult female is normally about half an inch long, exclusive of the ovipositor, which about equals the body in length. It is of the characteristic pimpline appearance, long and slender, black in color, with the legs red and the membranous portions of the venter white. The ovipositor is straight for most of its length, but toward the tip curves somewhat ventrally. The male is somewhat shorter and more slender than the female, as is commonly the case in this group.

VARIATION IN SIZE

There is considerable variation in size, depending upon the abundance of suitable larval food, a few individuals of each sex of not more than half the normal dimensions having been reared. However, extremely diminutive individuals are usually males.

TECHNICAL DESCRIPTION

FEMALE.—Length 11 mm.; ovipositor 11 mm., curving slightly ventrally at the tip; abdomen about twice as long as thorax. Head and abdomen black; tegula and a small triangular spot on the dorso-posterior angle of the mesonotum pale yellow, and a very small spot on the dorsal border of the mesopleurum dark brown; thorax otherwise black; palpi pale; antennæ with two basal segments black, remaining segments dark brown; all legs uniform dark fulvous; wings slightly brownish; veins and stigma brown. Thorax finely and sparsely punctate; propodeum more coarsely and densely punctate, with a shining, impunctate, median depression; abdominal segments coarsely and densely punctate; segments 2 to 5 with a smooth, shining, impressed area on the posterior lateral angle. Sheath of ovipositor black, densely hairy; ovipositor proper brown, shining.

MALE.—Length 9.5 mm.; more slender; otherwise, except in sexual characters, like female.

DESCRIPTIONS OF THE THREE SPECIES TO WHICH THIS SPECIES HAS BEEN REFERRED

Calliephialtes messor (Grav.).

Calliephialtes messor Gravenhorst was originally described in the genus Ephialtes in 1821 (1)[1] from a unique female from Russia. Dalla Torre (5) credits Gravenhorst with having recorded *Tinea mellonella* as a host of this species, but this should be accredited to Taschenberg (2).

E. messor n.—Pedibus rufo-fulvis, tibiis posticis arcuatis. f. (aculeo longitudine corporis).

Statura, imprimis proportione et tuberculis segmentorum, haec species medium tenet inter antecedentem et sequentem; tibiis posticis arcuatis ab utraque differt.

Longitudo fere 7 linearum. *Caput* palpis fulvis. *Thorax* puncto parvo testaceo ad radicem alarum. *Alae* testaceo-hyalinae, stigmate et radio fulvis, radice et squamula stramineis, areola triangulari sessili. *Pedes* rufofulvi, postici tarsis fuscis,

[1] Figures in parentheses refer to "Literature cited," p. 235-237.

tibiis arcuatis, supra fuscentibus. *Abdomen* thorace triplo longuis, eoque paulo angustius, cylindricum, segmentis 3 et 4 latitudine paulo longioribus, 5–7 quadratis, omnibus tuberculis lateralibus subprominentibus. *Aculeus* longitudine corporis, terebra badia.

Unicam feminam Besser e Volhynia transmisit.

A translation of this description is given below.[1]

Calliephialtes comstockii (Cress.).

The only reference to *Calliephialtes comstockii* Cresson is the original description published in 1880 (4). The type was reared as a parasite of *Retinia comstockiana* Fernald. It was referred to the genus Ephialtes.

Ephialtes comstockii Cresson, n. sp.

FEMALE.—Black, shining; thorax smooth, very feebly punctured; metathorax smooth, rounded, with two abbreviated, longitudinal, feebly developed elevated lines on disk, slightly divergent posteriorly; tegulæ white; wings hyaline, subiridescent, nervures and stigma fuscous, the latter with a pale spot at base, areolet as usual; legs including coxæ bright; posterior tibiæ and tarsi black; abdomen about twice the length of the thorax, distinctly punctured; sides of the second and following segments tuberculated; first segment a little longer than broad, broadly excavated at base and slightly grooved on disk above; second segment longer than broad, widened posteriorly; third and fourth segments quadrate; remainder transverse; ovipositor as long as the body; length of body .33 inch.

HABITAT.—Ithaca, N. Y. Parasitic upon *Retinia comstockiana* Fernald.

Calliephialtes pusio (Walsh.).

Calliephialtes pusio Walsh was originally described in 1873 (3) in the genus Ephialtes without host record, this constituting the only reference to the species in literature.

Ephialtes pusio, n. sp.—♀. Differs from *gigas* ♀ as follows:

1. The size is 1/2 smaller. 2. The face is highly polished and scarcely punctate. 3. The metathoracic carinæ are obsolete, being represented only by a slightly impressed stria extending 1/3 of the way to the tip. 4. The carinæ of the first abdominal joint are entirely obsolete. 5. The relative proportions of the first 5 abdominal joints are quite different, 2–4 being equal in length and each twice as long as wide, and 1 about 1/4 shorter, and 5 a trifle shorter than 2–4. 6. The usual tubercles are obvious only on 3 and 4, and are much less prominent and round, not elongated. 7. The ovipositor is rather piceous than black. 8. The legs are pale rufous, all the sutures a little darker, but both trochanters of the front leg, and the outermost one in the middle and hind leg, are whitish; and in the front leg the tarsal tip, in the middle leg the exterior face of the tibia and the whole tarsus, and in the hind leg the extreme tip of the femur and the whole tibia and tarsus, are pale fuscous. 9. The wings are subhyaline. Length ♀ .60 inch; front wing ♀ .36 inch; length abdomen ♀ .42 inch; width abdomen ♀ .06 inch; ovipositor .85 inch.

[1] *E[phialtes] messor*, n. sp.—Feet rufo-fulvous, posterior tibiæ arcuate, female with the ovipositor as long as the body.

In habitus, especially in proportions and in the tubercles of the segments, this species stands midway between the preceding [i. e., *E. tuberculatus*] and the following [i. e., *E. manifestator*]; in its arcuate posterior tibiæ it differs from both.

Length about 7 lines. Head with the palpi fulvous. Thorax with a small testaceous spot at the base of the wing; wings testaceo-hyaline, stigma and radius fulvous, base and tegulæ stramineous, areolet triangular and sessile; legs rufo-fulvous; posterior tarsi fuscous; tibiæ arcuate, shading to fuscous above; abdomen three times as long as the thorax, and slightly narrower, cylindrical, segments 3 and 4 slightly longer than broad, 5 to 7 quadrate, all lateral tubercles subprominent; ovipositor as long as the body, terebra brown.

A single female sent by Besser from Volhynia.

METHODS AND APPARATUS USED IN PROPAGATION

The most convenient and successful cage devised, the one in use at present, is constructed as follows:

A glass cylinder about 6 inches in diameter and 10 inches long is laid on its side in a baseboard constructed to keep the cylinder from rolling. The back end is covered with cheesecloth held in place by rubber bands. The front is a frame about 12 inches square, over which is tightly stretched a piece of cheesecloth. This is held against the front of the cylinder by means of rubber bands stretched between nails at the side of the frame and the side of the baseboard, permitting access to the cage without actually removing the front frame, by simply pulling the frame down, as the rubber bands will stretch sufficiently to admit the hands.

The cage is almost equally lighted from all sides, and the cheesecloth at each end permits good circulation. It is very easy of construction and management and very easily cleaned. In addition, a parasite either dropping or crawling from the top of the cage almost invariably reaches the rack of codling-moth cocoons at the bottom. About 15 adult female parasites can be placed in one cage.

The racks in which the codling-moth larvæ were placed for spinning were of two kinds, depending on the use to which the larvæ were to be put. For ordinary propagation the common corrugated strawboard used in packing glassware was used. This was cut across the corrugations into strips about three inches long and five-eighths of an inch in width. This gives comfortable quarters in each cell for a single worm. These were placed on edge in small wooden boxes 3 inches long by 2½ inches wide and three-fourths of an inch deep. Worms placed on the racks crawled almost immediately into the cells and shortly spun up. One box at a time was placed in a cage with the adult Calliephialtes for parasitization.

For the detailed study of the life history of the parasite double slides of transparent celluloid were constructed. The celluloid was cut into strips three inches by five-eighths of an inch. These were held apart and the space between divided into seven cells of the proper size by small slips of cardboard one-tenth of an inch thick and held in place by being fastened with shellac to one of the celluloid strips. The whole was held together by small gummed labels pasted over the ends. Each cell was numbered on the cardboard slip preceding it. Each slide was also given a number, and the slides used in each experiment were grouped under a Roman numeral. In this way notes on the contents of any given cell could be definitely associated with the subject without any chance of confusion. With this device it was only rarely that accurate observations on the development and activities of the insects within the cells could not be readily made by transmitted light.

When not under observation, each slide was placed in a folder of dark paper which left only one edge exposed, and was filed with others of the same experiment in a shallow box constructed for the purpose.

Observations were as a rule made twice daily, in the early morning and in the late afternoon, the intervening time being considered, for the purposes of the notes made, as half a day.

It was found that a living worm within its cocoon would respond immediately to the stimulus if a needle was thrust through the bottom of the cocoon. This aided materially in the determination of the time at which oviposition of the parasite took place, since, with but one exception, the parasite was never known to deposit an egg without first killing the host larva.

The food supplied the parasites consisted of sweet liquids, such as sugar solution, dilute molasses, and strained honey. All of these substances were lapped up greedily by the parasites of both sexes.

REPRODUCTION

THE EXTERNAL SEXUAL APPARATUS

OVIPOSITOR.—The ovipositor (figs. 1, 2, and 3) is composed of five long slender pieces. The two outer ones are black and hairy, grooved longitudinally within, and form a tube or sheath surrounding the ovipositor proper. Next inside of this is a smooth chitinized piece, deeply grooved on the ventral side and terminating in a prowlike point. At its base it is forked, indicating that it is formed of two opposed pieces fused along their dorsal edges. Within this is a pair of very slender flattened pieces barbed at their tips.

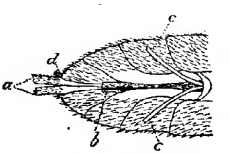

FIG. 1.—*Calliephialtes* sp.: Ventral view of terminal abdominal segments, showing relative position of elements of ovipositor. *a*, Valves of sheath; *b*, lance; *c*, lancets; *d*, cerci.

The outside pair together form the sheath. This has no part in the act of oviposition, but is merely a protection for the ovipositor proper, which is composed of the three other pieces. The single piece may be called the "lance," since it is with this that the host larva is pierced. The inner pair have been variously termed "lancets," "stylets," etc. In oviposition the egg passes down the channel formed by the three parts of the ovipositor proper.

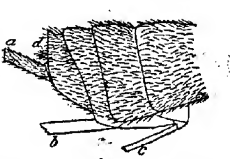

FIG. 2.—*Calliephialtes* sp.: Lateral view of terminal abdominal segments, showing relative position of elements of ovipositor. *a*, Valves of sheath; *b*, lance; *c*, lancets; *d*, cerci.

On each side and slightly above the base of the sheath is a small tuberclelike appendage bearing a number of long, stiff hairs. These are the cerci.

GENITALIA OF MALE.—The male external sexual organs (figs. 4 and 5) consist of two sets of paired pieces and the penis. The outer pair are

broad, tapering toward the tip, concave within, and, except during copulation, fit together like the two valves of a mussel shell, forming a sheath inclosing the other organs. They are homologous with the parts of the ovipositor sheath, and, like those, probably have no other function than that of protection for the more essential organs. The penis is probably homologous with the lance of the ovipositor, since its position in relation to the other organs corresponds to that of the lance in relation to the other portions of the ovipositor. It is a fleshy, flattened organ, terminating ventrally in two lobes contiguous at their apices. Immediately in front of these on the ventral side is an opening leading into the cavity of the organ. Immediately below the penis and on each side is a 2-jointed appendage corresponding to the lancets of the ovipositor. The basal joint of this organ is thick and muscular and on the dorsolateral side is prolonged into a blunt projection bearing at its tip a number of stiff hairs. It is probably a tactile organ, and may be called the genital palpus. The second joint is a large blunt tooth which curves laterad. It probably serves the double purpose of clasper and dilator. The genitalia, as described above, are surrounded at the base by a more or less cup-shaped chitinized piece, the cardo.

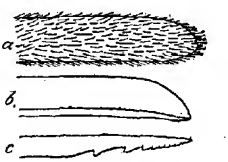

FIG. 3.—*Calliephialtes* sp.: Lateral view of tips of elements of ovipositor. *a*, Sheath; *b*, lance; *c*, lancet.

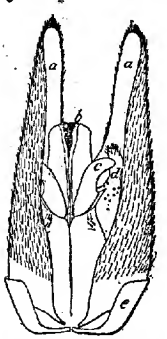

FIG. 4.—*Calliephialtes* sp.: Ventral view of male genitalia. *a*, Sheath; *b*, penis; *c*, clasper; *d*, genital palpus; *e*, cardo.

FIG. 5.—*Calliephialtes* sp.: Ventral view of clasping organ of male genitalia. *a*, Basal portion; *b*, clasper; *c*, genital palpus.

COPULATION

Copulation occurs shortly after the emergence of the female and may evidently be repeated. The attraction between the sexes seems to be rather weak and is somewhat stronger in the female than in the male, as evidenced by the excited movement of the antennæ and wings in that sex on the approach of the male. The male apparently must be within about an inch of the female before he becomes conscious of her proximity. Of courtship there is none, the male simply jumping to the back of the female as soon as he perceives her. If she is not ready for his attentions a lively encounter ensues, the female using her hind legs and wings in freeing herself from the male. The act of copulation is short, no case having been observed in which the sexes were together more than five minutes. In copulation

the tip of the abdomen of the male is curved down at one side of the abdomen of the female while he clings to her wings and body.

OVIPOSITION

Oviposition began in the cages about nine days after the emergence of the female. The stage of the host selected is the full-grown larva in its cocoon. In no case was any other stage attacked.

The act of oviposition (Pl. XX, figs. 2 and 3) was observed many times. The insect first explores the surface of the cocoon carefully with her antennæ. Then standing "on tiptoe" directly over the cocoon she raises the abdomen to a perpendicular position, at the same time lowering the ovipositor. Sometimes the ovipositor is lowered the entire distance free from the sheath, the latter remaining in line with the abdomen; but more frequently it is not released until it is at or below the horizontal, in which case the sheath bends downward, only the tip clasping the ovipositor. The sheath finally snaps back into position in line with the abdomen.

When the lowering of the ovipositor is completed it lies along the ventral surface of the abdomen and extends down between the legs, while the tip of the abdomen is bent downward over the base of the ovipositor. The tip of the ovipositor, guided by the antennæ, is placed against the surface of the cocoon. The antennæ are then extended in front of the head and almost parallel with the surface on which the insect is standing. The insect is now exactly analogous to a machine drill, the body and legs representing the machine and the ovipositor the drill. The bent-over tip of the abdomen is pressed against the base of the ovipositor, which bends forward against the ventral surface of the abdomen. With a more or less augurlike motion the ovipositor is forced through the cocoon. A few rapid jabs stir up the prospective host larva and it begins a desperate attack upon the ovipositor of its enemy, biting it and sometimes holding on with bulldog tenacity. In a number of cases the defense of the larva was so determined and powerful that the parasite was defeated and left the field minus a portion of her ovipositor, which had been bitten off by the larva. Usually, however, the parasite is successful in her efforts and finally thrusts her ovipositor into the larva, stinging it into insensibility. The stinging is usually repeated one or more times after intervals of rest. The subjugation of the host accomplished, the ovisitor is withdrawn from the host and thrust its entire length into the cocoon; then the parasite rests quietly for several minutes. In this position the abdomen is bent downward so that the tip is close to the base. The ovipositor sheath during all this time has retained its vertical position and is now in contact with the dorsal surface of the abdomen for about one-third of its length. In a few moments there begins a pulsation

of the membranous portion of the venter at the base of the ovipositor, at which time the egg is being forced into the ovipositor. The egg slips rather quickly down the ovipositor, becoming visible at a point just inside the cocoon and remaining visible during the remainder of its passage. It leaves the ovipositor, caudal pole first, at a point about 1 millimeter from the end on the ventral surface. It is placed at almost any point in the cocoon, not necessarily on the host larva.

Her egg having been deposited, the parasite usually gives a parting thrust or two and withdraws the ovipositor, which springs back into its sheath.

The duration of the act of oviposition is very variable, depending on the length of time required to locate and kill the larva. The shortest time observed was 11 minutes and the longest fully 45 minutes. The essential portions of the operation, however, probably do not require more than 4 or 5 minutes in the aggregate.

Only one egg is deposited at a time, and normally only one parasite develops on a single host. However, in a considerable number of instances superparasitism took place, and in a few cases under observation two parasites developed on a single codling-moth larva. This tendency was undoubtedly encouraged by the confinement of the cages, and as many as seven eggs were deposited in one cocoon.

No data were kept on the exact number of eggs deposited by individual parasites nor on the number deposited daily by individuals, since in each of the life-history cages from five to nine females were used. But the results in these cages indicate that the total individual oviposition was in the neighborhood of 75 eggs and the average daily oviposition about 2 eggs.

THE EGG

The egg (fig. 6) is opaque white, smooth, 1.5 mm. long, and about one-fifth as wide at the widest part. It is rounded at the cephalic end and tapers to a long point at the caudal end; in one plane it is considerably curved. The surface is without sculpture.

FIG. 6.—*Calliephialtes* sp.: Egg.

As the embryo develops, it draws away from the poles, and the chorion appears transparent and shriveled. Hatching takes place through a slit on one side near the cephalic pole, the larva freeing itself by a series of contortions which finally throw off the egg-shell, which is very tough and persistent.

The incubation period for 825 eggs was determined. It varied from one to seven days, depending on weather conditions. Table I shows the incubation periods by months, the number of eggs hatching in each period, and the weighted average mean temperature for each period and for the season.

TABLE I.—*Incubation periods of eggs of Calliephialtes sp. and the relation between incubation period and temperature at Vienna, Va., 1912.*

Incubation period.	Number of eggs hatching in—							Total.	Average mean temperature.
	Apr.	May.	June.	July.	Aug.	Sept.	Oct.		
									°F.
1 day	.....	16	4	49	17	1	.....	87	78.0
1.5 days	.....	68	36	75	57	4	10	250	74.4
2 days	.....	169	20	35	19	6	6	255	70.2
2.5 days	.....	15	7	7	1	13	29	72	67.0
3 days	.....	40	1	5	1	2	26	75	62.7
3.5 days	.....	2	.....	.....	.....	5	9	16	58.7
4 days	1	11	.....	.....	.....	5	7	24	58.9
4.5 days	.....	2	.....	.....	1	.....	9	12	55.3
5 days	8	4	.....	.....	1	.....	7	20	55.6
5.5 days	.....	.....	.....	.....	.....	.....	4	4	57.2
6 days	3	3	.....	.....	.....	.....	2	8	54.8
6.5 days	.....	.....	.....	.....	.....	.....	.....	.....	.....
7 days	2	.....	.....	.....	.....	.....	.....	2	53.2
Total	14	330	68	171	97	36	109	825	.....
Average	5.43	2.15	1.74	1.54	1.60	2.64	3.18	2.14	69.96

The relation of incubation period to the average mean temperature based on the figures of Table I is shown in graphic form in figure 7. Reference to this diagram will show that with a fair degree of constancy the duration of the incubation period varied inversely as the average mean temperature. The temperatures that are farthest from the curve (those of 3.5, 4.5, and 5.5 days) are based on the incubation periods of few eggs (16, 12, and 4, respectively), and the possibility of error was therefore greater than had the number been larger.

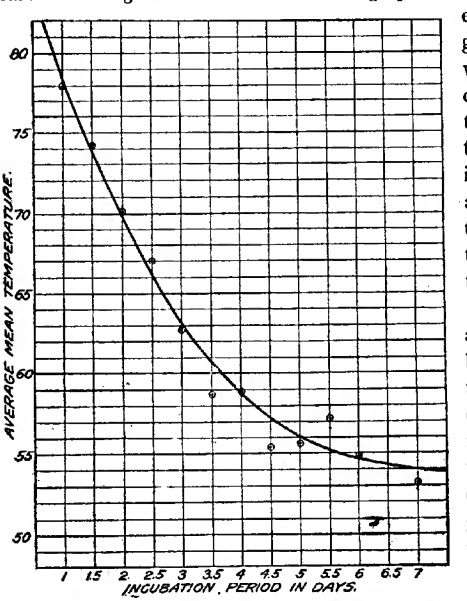

FIG. 7.—Diagram showing relation between incubation period of eggs of *Calliephialtes* sp. and average mean temperature at Vienna, Va., 1912.

THE LARVA

The newly hatched larva (fig. 8) is yellowish, slightly shorter than the egg, and widest across the head. The head is distinctly separated from the rest of the

body. The body is about three and one-half times as long as the head and is composed of 13 segments, tapering in size toward the caudal end. The head of the newly hatched larva is shown in ventral view in figure 9.

The form of the larva changes after the first molt to thick spindle shape; it is curved dorso-ventrally and is without a definite head. When full grown (fig. 10), it varies much in size, depending on the condition and abundance of food. Normally it is about three-eighths of an inch long and slightly less than a third as thick in its greatest diameter. It is pinkish white in color, the body contents showing through the transparent skin, while the adipose tissue appears as opaque-white granules. Larvæ that later develop into females average somewhat larger than those that develop into males. The face of the full-grown larva is shown, much enlarged, in figure 10, *b*.

FIG. 8.—*Calliephialtes* sp.: Dorsal view of newly hatched larva.

FIG. 9.—*Calliephialtes* sp.: Ventral view of head of newly hatched larva.

The larva begins feeding very shortly after hatching and may attack its host at almost any point, although it is more likely to attack the dorsum or sides than the venter. As feeding continues, it may change its position occasionally. In most cases the point of attack is finally shifted to a point near the posterior end of the host, the parasite pushing the collapsing skin up toward the head until there is nothing left of the host but a pellet consisting of skin and head shield. This is finally pushed to one end of the cocoon.

Calliephialtes is normally a solitary parasite, but as indicated in the foregoing discussion of oviposition, more than one egg was deposited on numerous occasions on a single host; though on only a few occasions did more than one live beyond the first stage. Usually the extra eggs did not hatch, owing probably to their being destroyed by the first larva to hatch. The actual destruction of eggs in this way was observed on a few occasions. However, in a very few instances, two larvæ developed on a single host. In such cases neither of the larvæ attained normal size and all produced dwarf adults. In only one instance of double parasitism was an adult female produced, and then the other individual was a male.

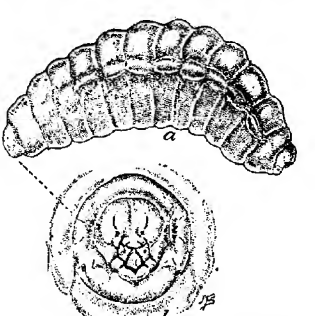

FIG. 10.—*Calliephialtes* sp.: *a*, Full-grown larva; *b*, face.

As a rule, the cocoon was started very shortly after the larva finished feeding, and for the purpose of this paper the beginning of the cocoon is taken as the end of the feeding period. However, in a considerable

number of cases some time elapsed after the larva had finished feeding before it began its cocoon, and in a few instances in which the insect was reared to maturity no cocoon was made. But such cases as that last mentioned resulted in diminutive adults. In most cases in which the cocoon making was delayed the supply of food had been small.

The feeding period—determined, as indicated above, from the hatching of the egg to the beginning of the cocoon—varied, in a total of 579 cases observed, from 3½ to 18½ days, with an average of about 7¼ days. In Table II all of the larvæ carried through to the spinning of the cocoon are recorded, the months in which they spun their cocoons and their feeding periods being indicated. The weighted average feeding periods for each month and for the entire period are also shown. This is undoubtedly higher in each case than the normal average, because, while the conditions of nature were imitated so far as possible in the cages, abnormal influences affected some of the larvæ so that not only was their feeding period protracted, but some time passed after they had finished feeding before they started their cocoons. However, it is impossible to tell at what point to begin eliminating such larvæ from the averages, so all are included.

TABLE II.—*Actual and weighted average feeding periods of larvæ of Calliephialtes sp. for the period from May to October and the average for the season at Vienna Va., 1912.*

Feeding period.	Number of larvæ in—						
	May.	June.	July.	Aug.	Sept.	Oct.	Total.
3.5 days			3	2			5
4 days			14	7			21
4.5 days		1	19	8			28
5 days		7	45	37			89
5.5 days	7	32	20	12			71
6 days	28	16	21	7			72
6.5 days	18	26	13		1		58
7 days	14	16	14	2	2	1	49
7.5 days	7	14	2				23
8 days	8	12	2	2	3	7	34
8.5 days	5	8			1	1	15
9 days		2		2	3	3	10
9.5 days	1	4			1	5	11
10 days	5		1		2	10	18
10.5 days		1				9	10
11 days	1	1	2			17	21
11.5 days						4	4
12 days	1		1			9	11
12.5 days						1	1
13 days		1				4	5
13.5 days		1				2	3
14 days						5	5
14.5 days		1				2	3
15 days						4	4
15.5 days						4	4
16 days						2	2
16.5 days						1	1
18.5 days						1	1
Total	95	143	157	79	13	92	579
Average feeding period (days)	6.98	6.85	5.54	5.21	8.42	11.53	7.07

A considerable portion of the larval life of Calliephialtes is passed in the cocoon. This period was determined for 116 female larvæ and 404 male larvæ. The females, after spinning their cocoons, required, on the average, about 2½ days longer to attain the pupal stage than did the males. This is probably somewhat less than the difference that would exist under natural conditions, inasmuch as the males under observation were somewhat more inclined to extend this portion of their development beyond the normal than were the females.

In Table III are brought together the data on that portion of the larval life passed within the cocoon. The figures include the prepupal period, which, not being a definite stage in the development of the insect but a transition stage, it is impossible to determine exactly. From this table are eliminated the data on 8 females and 11 males that remained in this condition for an abnormally long time. The actual maximum period recorded for females was 24 days and for males 36½ days.

TABLE III.—*Larval period of both sexes of Calliephialtes sp. in cocoon in various months, weighted average period for each month and for the season, and weighted average mean temperature for each period and for the season at Vienna, Va., 1912.*

Larval period in cocoon.	Females: Number of larvæ pupating in—				Total number of females.	Average mean temperature for period.	Males: Number of larvæ pupating in—				Total number of males.	Average mean temperature for period.
	May and June.	July.	Aug.	Sept.			May and June.	July.	Aug.	Sept.		
						° F.						° F.
4 days	....	....	....	....	....	.....	....	1	1	....	2	78.0
4.5 days	....	....	....	....	....	.....	....	2	1	....	3	78.2
5 days	....	....	....	....	....	.....	....	11	1	....	12	76.5
5.5 days	....	....	....	....	....	.....	1	9	2	....	12	74.8
6 days	....	1	....	....	1	77.8	11	34	24	1	70	74.2
6.5 days	....	1	....	....	1	77.7	13	19	13	....	45	72.7
7 days	1	1	....	....	2	72.3	20	23	20	4	67	71.0
7.5 days	1	3	....	....	4	74.4	17	8	11	1	37	72.4
8 days	8	3	1	....	12	72.2	23	10	14	9	56	71.7
8.5 days	4	3	....	....	7	73.9	22	....	5	....	27	67.7
9 days	11	1	3	....	15	70.9	10	2	10	2	24	69.6
9.5 days	7	....	2	2	11	69.6	7	1	1	....	9	68.5
10 days	8	....	2	1	11	68.4	5	1	2	3	11	71.1
10.5 days	6	....	....	....	6	67.5	1	....	2	1	4	70.9
11 days	16	1	1	4	22	68.8	2	3	....	2	7	72.4
11.5 days	4	....	....	....	4	69.0	3	1	1	2	7	72.1
12 days	4	....	1	....	5	69.1	....	....	....	....	....	.....
12.5 days	....	....	....	....	....	.....	....	....	....	....	....	.....
13 days	4	....	....	....	4	67.7	....	....	....	....	....	.....
13.5 days	1	....	....	....	1	66.8	....	....	....	....	....	.....
14 days	2	....	....	....	2	67.6	....	....	....	....	....	.....
Total	77	14	10	7	108	.....	135	125	108	25	393	.....
Weighted average period, days	10.2	8.0	9.7	10.4	9.9	.....	7.9	6.6	7.2	8.7	7.4	.....
Average temperature, ° F.	....	....	....	....	....	70.1	....	....	....	....	....	72.1

The figures of Table III are expressed in graphic form in the diagram (fig. 11), which shows the relation between temperature and the larval period in the cocoon. From the curve for males it is evident that individuals which took more than 8½ days between the spinning of the cocoon and pupation were more largely influenced by external conditions other than temperature than were those that required less time. The same is true of the females after 10½ days, although these showed the effect to a less marked degree than did the males.

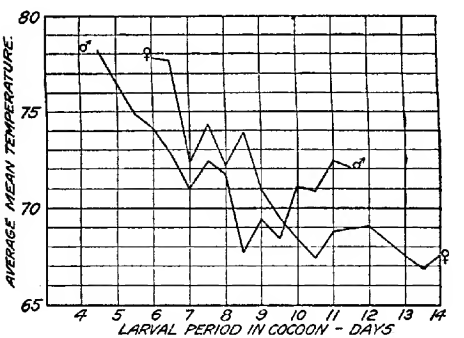

FIG. 11.—Diagram showing relation between temperature and larval period of males and females of *Calliephialtes* sp. in the cocoon at Vienna, Va., 1912.

It will be seen from the figures given for the feeding period and the larval period in the cocoon that the minimum and maximum possible total larval periods would be for females 9.5 and 42.5 days, respectively, and for males 7.5 and 55 days. The actual minimums and maximums were for females 12 and 27 days, respectively, and for males 7.5 and 51 days.

In Table IV are summarized the data obtained on the total larval period, with the exception of those on 13 females and 16 males in which this portion of the life cycle was unduly protracted. The total number for which the duration of this period was determined was 99 females and 344 males. The females required, in the average, nearly three days more to complete their larval life than did the males.

TABLE IV.—*Summary of data on total larval period of Calliephialtes sp. at Vienna, Va., 1912.*

Total larval period.	Females: Number of larvæ pupating in—				Total number of females.	Males: Number pupating in—				Total number of males.
	May and June.	July.	August.	September.		May and June.	July.	August.	September.	
7.5 days							1			1
8.5 days								1		1
9 days								1		1
9.5 days							1	1		2
10 days							10	2		12
10.5 days							8	4		12
11 days						1	19	8		28
11.5 days						5	15	3	1	24

TABLE IV.—*Summary of data on total larval period of Calliephialtes, at Vienna, Va., 1912*—Continued.

Total larval period.	Females: Number of larvæ pupating in—				Total number of females.	Males: Number pupating in—				Total number of males.
	May and June.	July.	August.	September.		May and June.	July.	August.	September.	
12 days	.....	2	1	.....	3	10	25	15	4	54
12.5 days	.....	3	1	.....	4	12	4	4	1	21
13 days	1	3	.....	.....	4	9	7	16	2	34
13.5 days	2	1	.....	.....	3	18	3	.....	2	23
14 days	2	.....	.....	.....	2	11	5	8	1	25
14.5 days	5	.....	.....	.....	5	18	.....	1	1	20
15 days	7	1	.....	2	10	10	2	5	1	18
15.5 days	6	.....	.....	1	7	5	.....	2	.....	7
16 days	4	.....	1	.....	5	13	1	4	2	20
16.5 days	10	.....	1	.....	11	6	.....	1	.....	7
17 days	3	1	1	2	7	2	.....	2	1	5
17.5 days	9	.....	.....	.....	9	1	2	.....	.....	3
18 days	6	.....	.....	.....	6	3	1	.....	.....	4
18.5 days	2	.....	1	.....	3	2	1	.....	.....	3
19 days	3	.....	.....	.....	3	1	.....	.....	.....	1
19.5 days	4	.....	.....	.....	4	1	.....	.....	.....	1
20 days	.....	.....	.....	.....	.....	1	.....	.....	.....	1
Total	64	11	6	5	86	129	105	78	16	328
Average period, days	16.5	13.3	15.4	15.9	16.0	14.3	11.9	12.8	13.6	13.2

THE PREPUPA

A few days before pupation the larva begins to show the constriction between the thorax and the abdomen, the eyes become discernible as distinct red spots, and before pupation actually takes place the appendages can be indistinctly seen through the delicate larval skin. The antennæ are coiled under the head instead of being extended along the venter, as in the pupa. In the prepupal stage (fig. 12) the sex of the insect can with certainty be determined for the first time. In the female prepupa the tip of the abdomen is bent slightly backward, indicating the developing ovipositor, while in the male the caudal segment is straight.

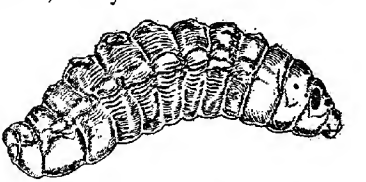

FIG. 12.—*Calliephialtes* sp.: Prepupa of female.

THE PUPA

When pupation takes place, the larval skin splits along the median dorsal line over the top of the head and for a short distance down the back, and through this opening the pupa makes its exit. Figure 13 shows the beginning of pupation of a female Calliephialtes. The rent in the exuvium, through which the antennæ are shown to extend, was

probably caused accidentally in the preparation of the specimen. By a series of twisting contortions the exuvium is gradually worked backward to the tip of the abdomen, where it is thrown off. It is very delicate and transparent, but as it is pushed back and becomes wrinkled it gradually appears darker until, when it is entirely shed, it is light grayish brown and is a mere shred.

In the male this is the end of the act of pupation, but it leaves the female with the ovipositor only a small fraction of its ultimate length and very thick.

The extension of the ovipositor is accompanied by a series of rythmical movements, about seven to the minute, during which the organ is repeatedly pressed against the dorsum of the abdomen. Whether the pressure thus exerted is the cause of the lengthening of the ovipositor or the effect of pressure from within the body and merely incidental could not be determined.

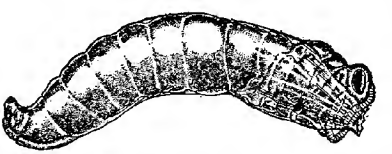

FIG. 13.—*Calliephialtes* sp.: Beginning of exuviation of female pupa.

The act of exuviation required about 15 minutes, but where the extension of the ovipositor was observed and timed the extension consumed from 35 to 41 minutes. The pupation of the male therefore required about 15 minutes, while the female required from 50 to 56 minutes to complete the process.

The newly formed pupa is entirely white, with the exception of the eyes, which are red. The legs and antennæ lie fully extended along the sides and venter, and in the female the ovipositor lies along the dorsum, extending the whole length of the body and curving somewhat at its tip over the head.

FIG. 14.—*Calliephialtes* sp.: Pupa of female and tip of abdomen of male pupa.

Gradually the eyes darken, becoming very dark before the adult color begins to appear over the rest of the body. The head and thorax are the next to begin to assume color, then the dorsal and ventral plates of the abdomen, the antennæ, the legs, and finally the ovipositor. When the coloring is complete (see fig. 14), the head, thorax, and antennæ are black, the eyes dark reddish brown, the wing pads gray, the chitinized portions of the abdomen and ovipositor nearly black, the legs yellowish, and the unchitinized portions white.

The pupal periods of 109 females and 366 males were determined. The average female spent 1.66 days longer in this stage than did the average male. This difference would, however, probably be somewhat greater

under natural conditions, as the males under observation were considerably more likely to extend this period beyond the normal than were the females. The actual difference is probably more closely indicated by the shortest pupal period for each sex, which gives a difference of two days.

In Table V the data on the pupal period are summarized and the average mean temperature for the various periods given.

TABLE V.—*Summary of data on duration of pupal period of Calliephialtes sp. and average mean temperature at Vienna, Va., 1912.*

Pupal period.	Females: Number transforming in—				Total number of females.	Average mean temperature.	Males: Number transforming in—				Total number of males.	Average mean temperature.
	June.	July.	Aug.	Sept.			June.	July.	Aug.	Sept.		
						°F.						°F.
6 days								1	1		2	77.2
6.5 days								1			1	79.0
7 days								17	8	3	28	78.2
7.5 days							1	2	5	9	17	74.7
8 days		2	1		3	78.7	2	11	9	23	45	74.4
8.5 days		1	1		2	72.4	4	8	11	14	37	73.4
9 days		2	2	5	9	76.9	17	9	14	21	61	68.4
9.5 days		3		1	4	72.6	22		14	1	37	68.3
10 days	5	10		3	18	71.9	51		28	2	81	68.0
10.5 days	7	6	1	4	18	71.6	18		16		34	65.3
11 days	10	1	2		13	69.9	19		1		20	66.9
11.5 days	17				17	68.6	3				3	66.4
12 days	15				15	68.9						
12.5 days	5	1			6	67.5						
13 days	4				4	66.7						
Total	63	26	7	13	109		137	49	107	73	366	
Average pupal period, days	11.50	9.90	9.57	9.73	10.78		9.94	7.83	9.19	8.36	9.12	
Average temperature, °F						70.9						70.3

The August column for males in Table V includes the data on 49 pupæ which were reared from unfertilized eggs. Whether the parthenogenetic character of these eggs had any effect in lengthening the pupal period is a question, but a comparison of the pupal periods of these with those of the 34 males that were developing at the same time from fertilized eggs shows that the pupæ from parthenogenetic eggs required a somewhat longer time. This is shown in Table VI. If these 49 individuals were eliminated in Table V, the total and average in the August column would be 58 and 8.61, respectively, and the grand total and grand average would be 317 and 9.0, respectively.

TABLE VI.—*Relative length of pupal stage of males of Calliephialtes sp. from fertilized eggs and those from parthenogenetic eggs at Vienna, Va., 1912.*

Pupal period.	Number of pupæ from—	
	Fertilized eggs.	Parthenogenetic eggs.
7.5 days	1	.....
8 days	1	.....
8.5 days	3	3
9 days	8	6
9.5 days	9	6
10 days	7	21
10.5 days	5	12
11 days	.....	1
Total	34	49
Average pupal period, days	9.44	9.87

The truth of the relation between the pupal period and temperature is in all probability not nearly so closely shown by the figures as is that between the incubation period and temperature by the figures in Table I, since the important factor of amount and condition of food has had opportunity to have its full effect. This factor, next to temperature, is probably the most important single factor influencing the duration of the stage, especially under the unnatural condition of the breeding cage.

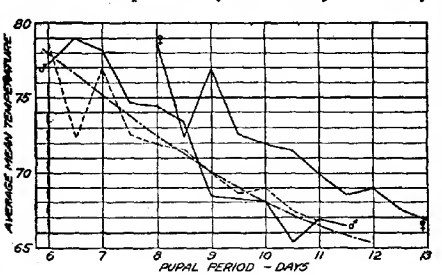

FIG. 15.—Diagram showing relation between pupal period of *Calliephialtes* sp. and temperature. The dot-and-dash line is the curve of average temperature, while the dotted line represents the female curve superimposed on that of the males at Vienna, Va., 1912. The greater tendency of the males to delay transformation to the adult stage is shown by referring the male and female to the line of average temperatures.

The figures of Table V are expressed in graphic form in figure 15.

THE COCOON

As stated, the larva usually begins its cocoon shortly after having finished feeding. Before starting its spinning it pushes the remains of its host to one end of the host cocoon and then accommodates its own cocoon to the size and shape of the space remaining within that of the host. The parasite cocoon therefore varies considerably in shape. It is usually, however, about one-half inch in length, about a third as broad,

and of the depth of the host cocoon. The upper and lower sides and the end next the remains of the host, most frequently the cephalic end, are flattened, while the edges and the other end are more rounded.

The cocoon is of a pale pinkish brown color, semitransparent, and is composed of a thin tissuelike material containing but few threads.

It was found very easy to observe by transmitted light the development of the parasite in its cocoon.

The period in the cocoon includes a part of the larval life, all of the pupal period, and a small portion of the adult life. By adding the minimums and maximums for each of these phases of development the total possible minimum period would be for females 14.5 days and for males 10.5 days and the total possible maximum period for females 39 days and for males 50 days. The actual minimum and maximum for females were 15.5 and 37.5 days, respectively, and for males 11.5 and 36 days.

The duration of this period was determined for 111 females and 396 males. The weighted average duration of this portion of the life history indicates that the females remain in the cocoon about four days longer than do the males. Table VII summarizes the data obtained. Eight females and five males, the recorded periods of which were far in excess of the normal for the month in which they emerged, are omitted from the table.

TABLE VII.—*Summary of period spent by Calliephialtes sp. in cocoon, showing number for each period by months, total for each period and each month, and average period for each month and for the season at Vienna, Va., 1912.*

Period in cocoon.	Number of females emerging in—				Total number of females.	Number of males emerging in—				Total number of males.
	June.	July.	Aug.	Sept.		June.	July.	Aug.	Sept.	
11.5 days								1		1
12 days										
12.5 days							1			1
13 days							10	1		11
13.5 days							4	1		5
14 days							4			4
14.5 days							2	1		3
15 days							9	6	6	21
15.5 days		1			1		3	6	5	14
16 days		1			1	2	5	16	15	38
16.5 days		1			1			11	11	22
17 days		2			2	8	3	29	18	58
17.5 days						13	3	20	7	43
18 days		2			2	26	2	17	5	50
18.5 days			1		1	19	1	4	2	26
19 days		2	1	1	4	20		7	3	30
19.5 days		3	1	4	8	12	1	5		18
20 days	2	2	3	1	8	11	1	2	3	17
20.5 days	2	4		1	7	4	1	1	1	7
21 days	6	1		4	11	8		1	1	10

TABLE VII.—*Summary of period spent by Calliephialtes sp. in cocoon, showing number for each period by months, total for each period and each month, and average period for each month and for the season at Vienna, Va., 1912*—Continued.

Period in cocoon.	Number of females emerging in—				Total number of females.	Number of males emerging in—				Total number of males.
	June.	July.	Aug.	Sept.		June.	July.	Aug.	Sept.	
21.5 days	8	1	.....	.....	9	2	.....	.....	.....	2
22 days	13	1	.....	1	15	1	.....	1	1	3
22.5 days	4	1	.....	.....	5	1	.....	.....	.....	1
23 days	11	1	1	1	14	2	.....	.....	.....	2
23.5 days	3	.....	.....	.....	3	1	.....	.....	.....	1
24 days	1	.....	.....	.....	1	1	.....	.....	.....	1
24.5 days	3	.....	.....	.....	3	2	.....	.....	.....	2
25 days	3	.....	.....	.....	3	.....	.....	.....	.....	.....
26 days	2	.....	.....	.....	2	.....	.....	.....	.....	.....
26.5 days	1	.....	.....	.....	1	.....	.....	.....	.....	.....
27 days	1	.....	.....	.....	1	.....	.....	.....	.....	.....
Total number	60	23	7	13	103	133	50	130	78	391
Average period, days	22.6	19.4	20.0	20.5	21.5	19.4	15.3	17.2	17.1	17.7

THE ADULT

Transformation from the pupa to the adult within the cocoon takes place one or two days before the emergence of the adult, depending largely on the difficulty encountered by the insect in biting its way by the remains of the host and through the two cocoons. The female effects her escape in a somewhat shorter time than the male.

In the spring the males appear some time ahead of the females, as indicated by the emergence of unforced material in the spring of 1912. From this material the first males appeared on April 23 and the first females 10 days later. In fact all but a few belated males appeared before the first female.

The males far outnumbered the females throughout the period covered by the observations, and it was found that the proportion of males increased with each succeeding brood. It appears that the effect of the unavoidably unnatural conditions of the artificial propagation tended to the production of males and that this effect was cumulative. Of the 528 individuals reared from mated females in the regular life-history experiments 396, or exactly three-fourths, were males. Table VIII summarizes the data on this point.

TABLE VIII.—*Proportion of sexes of Calliephialtes sp. from bisexual reproduction at Vienna, Va., 1912.*

Brood.	Number of females.	Number of males.	Ratio of females to males.
Hibernating	21	52	1:2.48
First	82	153	1:1.87
Second	20	112	1:5.60
Third	9	79	1:8.79
Total	132	396	1:3.00

Of the 57 individuals reared from parthenogenetic eggs all were males.

No definite data were obtained on the longevity of the females, for the reason that it was necessary to use all such in propagation experiments, and the individuals could not be distinguished. Some information on this point can, however, be obtained from the notes on the propagation cages. All females were fed, and hence there are no data on longevity without food.

Of the unforced hibernating females the first emerged on May 3 and the last on May 13. The latter was a weak individual and lived only 10 days. The last to emerge previous to it appeared on May 7. The earliest death, with the exception mentioned above, occurred on June 4 and the last on June 22. This gives a maximum longevity of 50 days, a minimum of 22 days, and an average of 36 days.

Females emerging from June 13 to 17 died from July 9 to August 7. The maximum longevity was 55 days, the minimum 22 days, and average 38.5 days.

Females emerging from June 24 to 26 died from July 19 to August 9. The maximum longevity was 46 days, the minimum 23 days, and the average 34.5.

Females emerging from June 27 to July 1 died from July 9 to 30. The maximum longevity was 33 days, the minimum 8 days, and the average 20.5 days.

The females surviving on August 9 in all first-generation cages were assembled in one cage on that date. Of these, 4 were from a lot emerging from June 18 to 20, an average of 51 days previous to the transfer; 3 from a lot emerging from June 22 to 23, an average of 47.5 days previously; 3 emerging from July 3 to 10, 33.5 days previously. The 10 females, after being placed together, died August 13 to 19, an average of 7 days later. The average longevity of the females from the earliest of the three lots was therefore 58 days, of those from the second lot 54.5 days, and of those from the third lot 40.5 days.

The average longevity of all females listed above was 51 days.

A number of surplus males emerging from June 14 to 22 were used in an experiment to determine the longevity with and without food. Of the 51 males used in the experiment 22 were fed and 29 unfed. For the fed males the maximum longevity was 51.5 days, the minimum 8.5 days, and the weighted average 32.5 days. The longest lived unfed male lived 10 days, the shortest lived 3 days, and the average lived 5.4 days. The average fed male therefore lived almost exactly six times as long as the average unfed male.

The adult Calliephialtes were very easily handled on account of their great docility. On many occasions while photographing the females in the act of oviposition the writer has carried a transparent slide on which a female was perched from the insectary to a greenhouse 20 feet distant, set it up in front of the camera, and made one or more exposures without the insect withdrawing her ovipositor; and in no case was the insect sufficiently disturbed to cause her to fly away.

The adults fed greedily at all times on the sweet liquids supplied them, and the males confined their feeding to this sort of diet. But the females very frequently fed on the juices of the codling-moth larvæ. This food they secured by repeatedly jabbing with their ovipositors the larvæ in the cocoons and licking up the juices that saturated the cocoon. Frequently a half or more of a larva would be consumed in this way, the parasite continuing to feed for an hour or more, alternately pumping the juices of the larvæ out with her ovipositor and licking them up. On one occasion a female Calliephialtes was observed to have killed and partially eaten a larva that had left its cocoon and was at large in the cage.

The total developmental period from oviposition to emergence was determined for 112 females and 399 males. For females it ranged from 23.5 days to 44.5 days and for males from 18 to 44 days. Both of the maximums as well as a considerable number of other records are based on individuals which, for some cause—usually inadequate food supply—were unable to go through their development in as short a time as they would have done under normal conditions. The records for 12 such females and 22 males are omitted from Table IX, which summarizes the data on the 100 other females and 377 other males. This table indicates that the average female required about 5 days longer to complete development than did the average male, the shortest period for females being 5½ days longer than the shortest for males.

TABLE IX.—*Total developmental period of Calliephialtes sp.; summary of duration of period by months, sexes, and for the season at Vienna, Va., 1912.*

Total developmental period.	Number of females emerging in—				Total number of females.	Number of males emerging in—				Total number of males.
	June.	July.	Aug.	Sept.		June.	July.	Aug.	Sept.	
Days.										
18	.....	.....	.....	.....	.....	.....	1	.....	.....	1
18.5	.....	.....	.....	.....	.....	.....	1	.....	.....	1
19	.....	.....	.....	.....	.....	.....	.....	1	.....	1
19.5	.....	.....	.....	.....	.....	.....	2	1	.....	3
20	.....	.....	.....	.....	.....	.....	2	1	.....	3
20.5	.....	.....	.....	.....	.....	.....	9	1	.....	10
21	.....	.....	.....	.....	.....	.....	11	4	2	17
21.5	.....	.....	.....	.....	.....	.....	3	3	4	10
22	.....	.....	.....	.....	.....	.....	5	5	5	15
22.5	.....	.....	.....	.....	.....	.....	2	10	11	23
23	.....	.....	.....	.....	.....	.....	3	14	14	31
23.5	.....	3	.....	.....	3	.....	1	18	13	32
24	.....	2	.....	.....	2	2	3	19	8	32
24.5	.....	.....	.....	.....	.....	.....	1	9	4	14
25	.....	2	1	1	4	10	.....	14	3	27
25.5	.....	.....	.....	.....	.....	10	.....	7	4	21
26	.....	2	3	2	7	14	.....	6	.....	20
26.5	.....	1	.....	2	3	17	1	3	3	24
27	.....	1	1	2	4	21	1	4	2	28
27.5	1	.....	.....	2	3	8	1	6	.....	15
28	.....	.....	.....	2	2	17	.....	4	1	22
28.5	3	2	.....	.....	5	2	.....	.....	.....	2
29	8	2	.....	.....	10	15	.....	.....	.....	15
29.5	6	.....	1	2	9	1	.....	.....	.....	1
30	8	2	1	.....	11	4	.....	.....	.....	4
30.5	6	.....	.....	.....	6	.....	.....	.....	.....	.....
31	6	1	.....	.....	7	2	.....	.....	.....	2
31.5	2	1	.....	.....	3	3	.....	.....	.....	3
32	2	2	.....	.....	4	.....	.....	.....	.....	.....
32.5	3	.....	.....	.....	3	.....	.....	.....	.....	.....
33	6	.....	.....	.....	6	.....	.....	.....	.....	.....
33.5	1	.....	.....	.....	1	.....	.....	.....	.....	.....
34	1	.....	.....	.....	1	.....	.....	.....	.....	.....
35	1	.....	.....	.....	1	.....	.....	.....	.....	.....
35.5	1	.....	.....	.....	1	.....	.....	.....	.....	.....
36	4	.....	.....	.....	4	.....	.....	.....	.....	.....
Total	59	21	7	13	100	126	47	130	74	377
Average developmental period, days	31.1	27.4	27.1	27.2	29.6	27.2	21.7	24.1	23.5	24.7

No definite experiments were conducted in experimental control of the development, but during the warm weather many strawboard slips of parasitized larvæ were placed in cold storage to retard the development of the parasites. In so far as it was possible to determine, they were placed in storage after the spinning of the parasite cocoon. This retardation of development had no apparent effect on the further development after removal from cold storage. It did seem, however, to reduce the activity and vitality of the resulting adults. L. J. Newman (18) records

the keeping of immature specimens of *Calliephialtes messor* in cold storage for a period of 14 months, after which they emerged without having suffered in the least.

SEASONAL HISTORY

The first females to emerge from hibernation in the spring of 1912 appeared on May 3 and the last on May 15. These were placed with males in propagation cages. The first egg was deposited on May 13, ten days after the first emergence.

In order to determine the maximum and minimum number of generations in a season, the five earliest and five latest appearing female progeny of the hibernating brood were used in the life-history cages, a separate cage being used for each group. The same plan was followed out with each succeeding generation. From the earliest female progeny three complete generations were reared, and from the latest group two generations were bred. With the hibernating brood this gives a maximum of four generations in the year and a minimum of three generations. Table X summarizes the data on the number of generations. It is interesting to note that the total time consumed by the three generations is only one day longer than that consumed by the two.

TABLE X.—*Number of generations of Calliephialtes sp. reared at Vienna, Va., 1912.*

Generation.	Maximum number of generations.		Minimum number of generations.	
	Date of first female.	Total cycle.	Date of last female.	Total cycle.
		Days.		*Days.*
Hibernating	May 3	.....	May 13	.....
First	June 13	41	July 13	61
Second	July 18	35	Sept. 12	61
Third	Sept. 3	47	.....	.....
Total period, days	.....	123	.....	122
Average cycle, days	.....	41	.....	61

Development ceased at about 50° F., although oviposition was frequently carried on actively at that temperature. After the middle of October very few eggs hatched, although the last eggs of the season were not deposited until November 1. All but a very few of the larvæ that hatched at this season passed through the feeding stage and constructed their cocoons.

Calliephialtes sp. hibernates as a full-grown larva in its cocoon. In this stage it is capable of withstanding a very low temperature. The mortality among hibernating larvæ during the winter of 1911–12 was very slight, if not nil, in spite of the fact that a temperature of −6°

Fahrenheit was recorded in the insectary. This is an unusually low record for the locality and indicates that the species would have no difficulty in acclimating itself were it liberated in the region.

ALTERNATE HOSTS

The female parasites appeared in the spring a few days in advance of the first adult codling moth, or somewhere about 40 days before they could, under natural conditions, attack the first brood of larvæ of the codling moth. The hibernating brood of parasites would therefore have passed the greater portion of their adult life before an abundance of codling-moth larvæ could be found. This would necessitate a very small first generation of the parasites unless they would attack some other host.

To determine if Calliephialtes would attack other species of insects, larvæ of *Enarmonia prunivora* Walsh, *Euzophera semifuneralis* Walk., and *Gnorimoschema gallaesolidaginis* (Riley) were placed in the propagating cages with actively ovipositing female parasites. The larvæ of the first two species were placed in transparent cells, and those of the last were allowed to remain in their galls. Only a single Enarmonia larva was available, and this was parasitized within 2 days, a diminutive male Calliephialtes emerging from the cocoon 22 days later. This species is, however, much smaller than the normal full-grown larva of the parasite, and it is doubtful if it would serve in the long run as an alternate host.

Of the two other species of larvæ neither was apparently given the least attention by the parasites, although those of Euzophera were left in the cage for several weeks.

Codling-moth larvæ containing the internally parasitic larvæ of *Ascogaster carpocapsae* were readily attacked and parasitized by Calliephialtes. This always resulted in the death of the earlier parasite and the production of a diminutive adult Calliephialtes.

On one occasion a Calliephialtes larva that had already spun its cocoon was attacked and killed by an adult of the same species. When the fact was discovered, a small living larva was feeding on the dead parasite larva. This parasite larva died without spinning.

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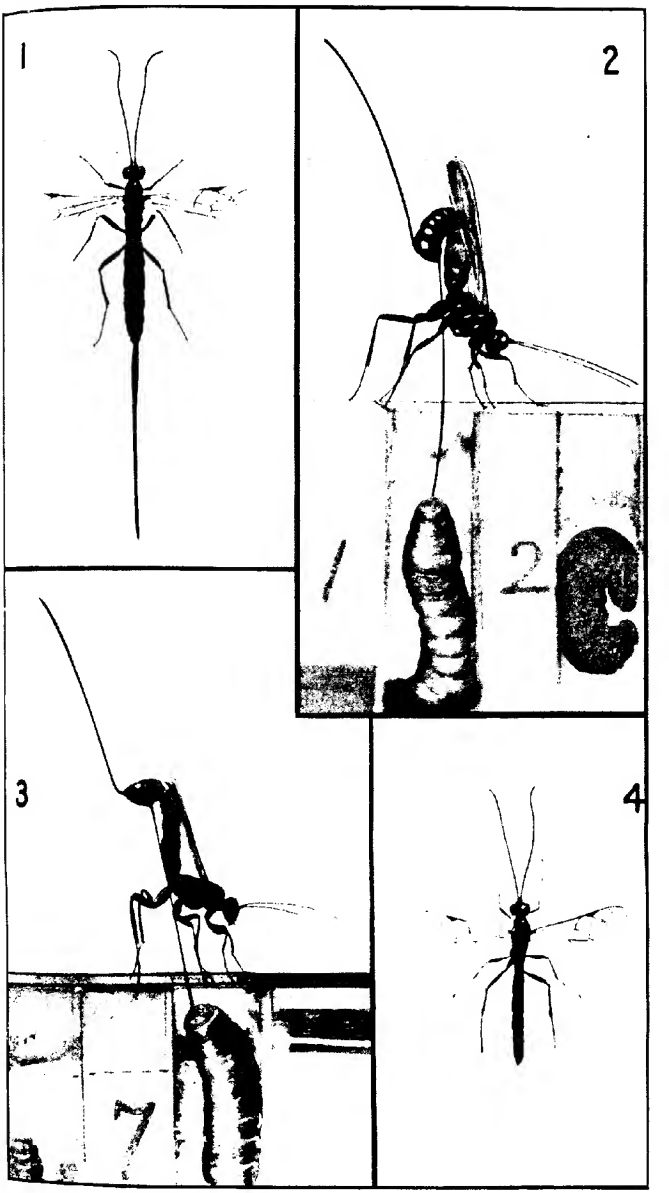
DESCRIPTION OF PLATE

PLATE XX. *Calliephialtes* sp. Fig. 1.—Female. Figs. 2 and 3.—Characteristic positions assumed by the insect in oviposition. Fig. 4.—Male. Figures 1 and 4 are enlarged about 2½ times. Figures 2 and 3 are retouched photographs from life; enlarged about 3 times.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POLYPORUS DRYADEUS, A ROOT PARASITE ON THE OAK

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Bulliard (1789, 1791) [1] figured and described under the name *Boletus pseudo-igniarius* a fungus which most European mycologists believe is the plant now called *Polyporus dryadeus*. Apparently the next record of this fungus is by Persoon (1799), where it is described as *Boletus dryadeus*. Again it is described by the same writer in his Synopsis Fungorum (1801), where Bulliard's fungus is listed as a synonym. It is first named *Polyporus dryadeus* by Fries (1821), who describes the plant and cites as synonyms the names given by Bulliard and Persoon. Hussey in Illustrations of British Mycology (1849) gives a fairly good figure of the sporophore and a most excellent mycological description of the fungus, with its habitat.

Since 1849, repeated references to this fungus are found in European mycological literature, but nothing was written concerning the rot produced by it in the oak until Robert Hartig in his epoch-making work on the true nature of the rots of woods (1878) described a heart rot of the oak which he attributed to *Polyporus dryadeus*. A careful study of Hartig's figures and the description of the sporophore which he found associated with the white heart-rot so accurately described by him is sufficient to convince anyone who is familiar with the true *P. dryadeus* that Hartig's fungus was not *P. dryadeus*. It is undoubtedly identical with the heart-rotting fungus known in America as *P. dryophilus* and found by Hedgcock (1910 and 1912) to be associated with a whitish piped rot in oak.

Polyporus dryophilus has one character, a hard, granular, sandstone-like core, that is unique and not possessed by any other polypore known to the writer. The sporophore of this plant, represented by numerous specimens collected by Hedgcock and the writer in western and southwestern United States, shows this hard, granular core exactly as figured and described by Hartig in his article on *P. dryadeus*. This core extends back some distance into the tree in oaks; it is usually irregularly cylindrical while in the tree, but on its emergence from the tree it swells into a tuberous or spheroid mass and finally occupies the central and rear part of the sporophore. (Pl. XXI, fig. 1.) If the sporophore is formed from a large branch hole, it is usually of the applanate type, with a small core, but when the sporophore forms directly on the body of the

[1] Bibliographic citations in parentheses in the text of this article refer to "Literature cited," p. 248.

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tree, as it usually does, the shape is tuberous, ungulate, or even subglobular (Pl. XXI, figs. 2 and 3), with the bulk of the sporophore composed of a hard, granular core. This core usually has white mycelial strands. (Pl. XXI, fig. 3.) The sporophore of *P. dryophilus*, therefore, has normally three distinct kinds of structures: (1) The hard, granular core, (2) the fibrous layer which surrounds this core except at the rear, and (3) the layer of tubes on the lower surface. Specimens are often found, however, especially from the western part of the United States, in which this fibrous layer may be entirely absent between the tubes and the granular core. (Pl. XXI, fig. 3.)

The sporophore of *Polyporus dryadeus* never has this granular core, but its context is fairly homogeneous and of a fibrous-corky structure. (Pl. XXI, fig. 4.) Another very important difference between the two species is the location of the sporophores on the host tree. In *P. dryadeus* the sporophores are always on the exposed roots or on the trunks at or very close to the ground. The reason for this is explained later in this article. In *P. dryophilus* the sporophores are higher on the trunk of the tree, and in some cases are on the branches.

The rot described and figured by Hartig is identical with the rot produced by *P. dryophilus*, but does not resemble in the least the rot produced by the real *P. dryadeus*. Since Hartig's time European mycologists have followed him in descriptions of the rot wrongly ascribed to *P. dryadeus*, but most of them have described the sporophores of the true *P. dryadeus* both as to its character and location on the tree—i. e., at the base of oaks. For instance, Von Tubeuf, in his Disease of Plants (1897), describes fairly well the sporophore of *P. dryadeus*, while his photograph of the rot is that of *P. dryophilus*. Massee, in his Diseases of Cultivated Plants and Trees (1910), states that "the largest specimens usually occur near the ground line, but it also springs from points where branches have died or been broken off." The latter statement, so far as can be ascertained by the writer, is incorrect as to the location of the sporophores of *P. dryadeus*, but is correct for *P. dryophilus*. Massee also quotes Hartig as to the character of the rot produced.

Polyporus dryophilus is known in Europe under at least three different names: *Polyporus fulvus* Fries (Pl. XXI, fig. 5), *P. friesii* Bresadola, and *P. vulpinus* Fries. (Pl. XXI, fig. 6.) According to Lloyd (1913), not only are *P. fulvus* Fries and *P. friesii* Bresadola synonyms for *P. dryophilus*, but the *P. corruscans* of Fries is also the same plant.[1] *Polyphorus vulpinus* is the name given to the form of *P. dryophilus* found on species of Populus, authentic specimens of which were seen by the writer at the New York Botanical Garden in collections from Finland and Sweden and also from

[1] Since this article was written, the writer, through the courtesy of Mr. C. G. Lloyd, has examined the specimens of *Polyporus corruscans* and of *P. rheades* deposited in the Lloyd Herbarium at Cincinnati, Ohio. Both of these plants as represented in this herbarium are *Polyporus dryophilus*, the former being the usual form found on oak and the latter the one occurring on poplar. According to Mr. Lloyd, the type of *P. rheades*, found by him in Persoon's Herbarium, is undoubtedly the plant called "*P. vulpinus*" by Fries.

Maine. In the Cryptogamic Herbarium of Harvard University there is a collection on *Populus grandidentata* Michx. from New Hampshire, while in the laboratory of forest pathology of the Department of Agriculture at Washington, D. C., there is a fine collection on *Populus tremuloides* Michx. from near Steamboat Springs, Colo. (Hedgcock, 1913).

This fungus on Populus agrees in all essential characters with the form of *Polyporus dryophilus* found on oak. The sporophores are, however, somewhat smaller than those usually found on oak and approach the applanate type. (Pl. XXI, fig. 7.) The hard granular core is always present, but is formed between the sapwood and bark (Pl. XXI, fig. 8), as the fungus is able to rot the sapwood as well as the heart of this host. It therefore does not have to depend on branch holes or other openings through the sapwood in order to form its sporophores, as it does in the oak.

Through the kindness of Von Tubeuf the writer obtained a European specimen of Hartig's so-called rot of *Polyporus dryadeus* in oaks. (Pl. XXII, fig. 1.)[1] It is unquestionably the rot produced by *P. dryophilus.* (Pl. XXII, fig. 3.)

The following discussion of the rot caused by *Polyporus dryadeus* embodies the results obtained from extensive field studies made in the forests of Arkansas, eastern Texas, Oklahoma, Maryland, and Virginia.

The sporophores of *P. dryadeus* are always found at or very near the ground at the base of the host. This first suggested to the writer that the fungus might be a true root-rotting organism. Trees with sporophores at their bases and wind-thrown oaks with and without the sporophores attached were carefully studied. Sections of the trees were cut, roots dug up and examined, and every effort made to determine exactly the character of the rot produced. The roots and stools of 20 trees attacked by this disease were examined, and sections of the various stages of the rot were studied.

The microscopic characters of the rot from each tree were found to be identical, although of the 20 trees examined 5 were in Arkansas, 3 in Texas, 2 in Oklahoma, 4 in Maryland, and 6 in Virginia. In every instance the trees were found to have a white rot which attacks first the sap and finally the heartwood of the roots. The rot originates in the lower portion of the roots and spreads in them toward the base of the tree.

The first evidence of the disease is a reddish brown discoloration of the inner bark and cambium. If the diseased roots are exposed in a damp chamber at this stage, white floccose spots of mycelium will appear on the outside of the bark, but the rot has not yet become evident in the wood. As the rot progresses, discolored, watery, reddish

[1] Figure 1 on Plate XXII was made from a photograph of a piece of the original type material used by Hartig in his description of the rot of *Polyporus dryadeus* (1878).

brown areas, which become hazel in color when the wood is dried, appear on the surface of the sapwood and in its outer layers. At this stage a cross section of the root has a mottled appearance, and this discoloration gradually spreads till the root is affected to its center. The earliest discolored spots have by this time turned white. (Pl. XXII, fig. 2.) Later, as the rot ages, especially in the larger roots which lie near the surface of the ground, this white changes to a cream and finally to a straw color. The lower portion of the smaller diseased roots, those 2 inches or less in diameter, become completely rotted and white throughout before the advancing rot has reached the stool of the tree. On these small rotted roots the bark separates easily from the wood, since much of the living bark has been destroyed. The bast fibers, however, remain intact, which gives the inner bark a loose, shredded appearance. The rot gradually moves up the roots till the stool is reached. This is also attacked by the fungus, but the rotted area ends abruptly at the surface of the ground.

A radial-longitudinal section of the rot in a fresh state has a sodden, watery appearance, with white longitudinal and transverse lines somewhat like the rot produced by *Polyporus hispidus* in oaks. These white lines or bands are not cellulose, however, but are spaces filled with air and the mycelium of the fungus in the region of the large vessels. When the rotted wood is thoroughly dry, these white lines disappear, and the uniformly creamy-white rot is left. The rot in all the trees examined did not extend for any appreciable distance into the heartwood of the trunk proper above the collar of the tree, even when the large, completely buried roots, 6 to 12 inches in diameter, were rotted throughout.

The thoroughly rotted wood when dry is very light in weight and, superficially, looks and feels like pith. If a freshly dug root in the advanced stage of the rot is twisted, it will split into concentric layers and also into longitudinal blocks, giving the broken end of the root a coarse, fibrous appearance. The lower ends of the diseased roots may be in a thoroughly rotted condition, easily splitting into these concentric layers and rough, fibrous masses, while that portion of the root next to the base of the tree remains comparatively sound. The roots of several of the trees overthrown by the wind were thus affected. The presence of this rot is often indicated by irregular white mycelial patches on the outside of the bark of the root or of the stool of the tree.

In a radial-longitudinal section through the heartwood of a diseased root the advancing line of the rot first appears as a watery dark-brown zone 1 to 3 inches wide. This dark area terminates rather abruptly in the ultimate cream-colored rot on one side and in the sound heartwood on the other. A microscopic examination of the diseased wood shows that the starch and other cell contents of the roots are first extracted; then the walls of the wood elements are gradually destroyed, especially the walls of the tracheids and vessels adjacent to the large medullary

rays. The bordered pits in the vessels are usually reduced to long, elliptical openings running transversely across the walls, and the bordered pits of the tracheids become large, round holes, which often coalesce, thus splitting the tracheids longitudinally. The pits of both large and small medullary rays are somewhat enlarged, while their radial and tangential walls are perforated with holes.

Even in the early stages of the rot, when the discolored spots are beginning to show in the sapwood of the roots, the vessels have colorless hyphæ in them, while in the later stages many of the vessels become filled with a mass of colorless hyphæ having filaments 4μ or less in diameter. The wood-parenchyma fibers show enlarged pits and perforated radial walls, and the pits in the wood fibers are also enlarged. The walls of the medullary rays are much corroded and often disappear entirely.

Only very slight evidence of delignification is shown by the chloriodid of zinc test. After standing 24 hours in this reagent there is a slight cellulose reaction in the walls of the vessels, tracheids, and wood fibers but none in the medullary rays. In making free-hand sections of the diseased wood the medullary rays and vessels are easily ruptured, owing to the thinning and weakening of the walls by the solvent action of the fungus.

The concentric splitting of the rotted wood usually occurs in the zone of the larger vessels, which are weakened by the corrosion of their bordered pits and walls. The longitudinal splitting is caused by the coalescence of the enlarged bordered pits of the tracheids and the thinned walls of the medullary rays. The discolored areas seen in the earlier stages of the rot are due to the presence in the cells of the medullary rays, wood parenchyma fibers, and sometimes in the lumen of the wood fibers of a brownish liquid, which disappears before the white stage of the rot is reached. In the final stage of the rot the wood is somewhat spongy in texture and when dry is easily crushed between the fingers.

Old sporophores were often found at different places on the collar of the diseased tree, due probably to the gradual rotting of the roots upward toward the stool of the tree and the formation of sporophores whenever a rotted area reached the collar of the tree or the underside of a root whose upper surface was exposed to the air. The sporophores are usually attached to the trunk of the tree at the surface of the ground, but they were also found on the exposed roots or even in rare cases on the ground, having been produced from hyphæ issuing through the soil from diseased roots lying a short distance below. Only one sporophore was found on the trunk at a distance above the collar of the tree, and in this case two trees had grown together at the butts for a distance of 12 inches. The rot had extended from the diseased roots upward in the injured sapwood of the oak along the juncture of the two trunks, and a small sporophore had formed 10 inches from the ground.

The sporophores when old and mature usually have a hard fibrous-corky to corky-woody context and a very rough, uneven, tuberculate upper surface, owing to the leaves, twigs, and other foreign substances falling on the upper surface of the growing pileus. (Pl. XXII, fig. 4.) After weathering for some months, the color of the pileus is a chestnut brown or sometimes becomes almost black and rimose. The old sporophores as a rule are partially destroyed by insects, especially the subhymenial layer and the adjacent ends of the pores. Portions of the outer pore surface, the central part of the context, and the base of the sporophores usually persist and can be found attached to the bases of the diseased trees for several years after maturity.

The mouths of the pores in the weathered sporophores are stuffed to a depth of 0.5 to 1 millimeter with a firm, brown mycelial mass, thus completely hiding all trace of the pores from a surface view. This stuffed pore layer becomes hard and brittle and gradually cracks in weathering and peels off from the deeper and more insect-eaten portion. Immature specimens shipped before being thoroughly desiccated have the tubes loosely stuffed with a delicate, white arachnoid mycelium, which appears on the spore surface as a thin creamy layer about 0.5 of a millimeter thick. This condition is probably due to a growth developed in the sporophore while in transit in a damp state. The stuffed mouths of the pores in old weathered sporophores is apparently a normal state of old specimens from certain sections of the United States. However, this stuffed condition of the pores in old sporophores is not always present, as several specimens both from America and Europe were seen by the writer in which the mouths were entirely free and open.

The tubes in all the specimens examined—both American and European—contain characteristic setæ. They are dark chestnut brown, thick walled, curved, cat's claw to hawk beaked in shape, giving them a somewhat bulbous-shaped base when seen in side view. They are 7 to 12μ thick at base, 15 to 24μ long, and usually project 10 to 20μ beyond the hymenial surface into the tube cavity.

The sporophores vary greatly in shape and size, ranging from 9 cm. long, 5 cm. wide, and 1½ cm. thick to 20 cm. long, 15 cm. wide, and 10 cm. thick, and may be simple or imbricated, depending to a great extent on the environment and food supply. In many of the thick sporophores growing from the collar of the tree the pore surface is borne at an angle of 40° to 60° to a horizontal plane. In the thinner and broader specimens the pore surface approaches more nearly the normal angle of other dimidiate sporophores. The margin is very thick and rounded in most of the specimens. The cavities left in the upper surface of the pileus by the drops of water which exude during the rapidly growing period of the sporophore are plainly discernible even in many of the old sporophores. The pore surface extends entirely to the point of attachment to the substratum even when the sporophore has a rounded substipe, as is often the case when it forms on the upper surface of exposed roots.

When sporophores are developed at the collar of trees growing in sandy land, the soil for 4 to 6 inches wide and 2 to 3 inches deep immediately at the base of the sporophore is often cemented into a hard, compact, bricklike mass, apparently by hyphæ, as many colorless fungous threads were found ramifying through it.

Polyporus dryadeus has been found attacking the roots of *Quercus texana* Buckl. and *Q. nigra* L. in eastern Texas. Some of the diseased trees were dying, while others were evidently in poor health. It has been found on *Q. alba* L. and *Q. velutina* Lam. in the Ozark National Forest, of Arkansas. The majority of the trees in the Ozarks affected with the disease caused by *P. dryadeus* were growing on sandy ridges and southern slopes where the soil was thin and conditions were unfavorable to rapid, vigorous growth. Two trees of *Q. minor* (Marsh) Sarg. were found with this disease in Oklahoma; one was dead and the other in apparently fair health.

Polyporus dryadeus also occurs in *Q. alba* L., *Q. rubra* L., and *Q. prinus* L. in Virginia, where seven trees were found with this rot; five were growing in crowded, unfavorable conditions, while one was standing at some distance from other trees and was apparently in good health. Yet at least two large roots of this lone tree—a white oak—were thoroughly rotted, while sporophores were found on three sides of the tree, one growing from the top of an exposed root. This sporophore was over 1 foot tall and at least as wide, judging from the old weathered remains. It was from this root that figure 5 of Plate XXII was taken. Of the five crowded trees one was much suppressed and would probably have died in a year or two. This tree was dug up, and studies were made of its roots, stool, and trunk. All of its roots, except three large lateral ones which ran near the surface of the ground, were completely rotted by *P. dryadeus*. The three living roots were partially rotted on the lower side and at the ends, but were still alive and strong enough to hold the tree in the ground. Old sporophores were found on all sides of this tree at the ground line.

The trees of *Quercus prinus* which were attacked by this root rot were found by Mr. G. F. Gravatt, of the Office of Investigations in Forest Pathology, who made the following statement concerning the diseased trees:

> Early in July at Bluemont, Va., three small trees of *Quercus prinus* were found which had been killed while in full leaf and which from a distance were mistaken for chestnut trees that had been girdled by the chestnut bark disease (*Endothia parasitica*). Whitish spots of mycelium were found on the bark of nearly every root, while the lower portions of the roots were so thoroughly rotted that the two smaller trees were easily pulled up by hand. The two small trees were somewhat suppressed, but the largest (3¼ inches in diameter) was situated in an open space in the woods. These three trees were about 100 yards distant from each other.

The writer examined the rot from the roots of these diseased trees and found that it was caused by *Polyporus dryadeus*.

Four trees of *Quercus alba* were found affected with this disease in Maryland. All had been uprooted by the wind, two very recently, so that the character of the earlier stages of the rot and its progress in the roots was easily observed. In both of these trees the rot was only in the lower ends of the roots and had not reached the stool nor formed sporophores. Three of these uprooted trees were growing in dense stands and were much suppressed.

Oaks which have been uprooted by the wind may be separated into two classes: (1) Those whose root system has been weakened by insect or fungous attack and (2) those with a very shallow root system, due to the presence of impermeable layers of rock in the subsoil or to the ground-water being constantly near the surface of the ground (within 1 to 2 feet). Trees uprooted by wind owing to rotten roots have very little soil adhering to the upturned stool of the tree, as most of the roots break off within 1 to 2 feet of the base of the tree. On the other hand a tree with a sound root system brings with it when uprooted a large mass of earth several cubic yards in size. Bearing this in mind one can often distinguish, even at a distance, wind-thrown trees with sound roots from those overthrown on account of root-rot.

In every instance where the sporophores of *Polyporus dryadeus* were found on trees the roots were diseased with the same type of root-rot. In wind-thrown trees where the disease was not far enough advanced to produce sporophores the rot was identical with that obtained from the roots of trees which had sporophores of *P. dryadeus*. The rot in such uprooted trees evidently began at some point on the lower end of the roots and advanced up the roots toward the base of the tree, stopping, however, when it reached the surface of the ground. Roots lying very near the surface of the soil, especially large ones with their upper surfaces exposed to the air, are not entirely rotted or even killed by this fungus. Many instances of such superficial roots were found in which the part underground was rotted while the upper portion remained alive. The cross section of the root illustrated in Plate XXII, figure 6, shows the upper part alive, while the lower and more deeply buried portion is rotted. This root forked some 2 feet from the tree; one root, 10 inches in diameter, went down deep in the soil and was thoroughly rotten and dead; the other fork was 2 to 4 inches deep and was perfectly sound 2 feet from where the rotted root joined it.

The inability of the fungus to rot exposed roots and the trunk above the surface of the soil, coupled with the further fact that the sporophores usually are attached to what superficially appears to be sound wood, probably explains why the connection between this rot and the fungus causing it has not been previously noted. Trees in all stages of this disease were seen; some were already dead, others dying, others on the decline, while some showed no evidence of the disease until they were overthrown by the wind and the decayed roots were exposed. Some of

the trees bearing sporophores were apparently in a healthy condition, yet an examination of the root system showed in every case one or more large roots completely rotted Two stumps of *Quercus alba* were found with sporophores of *Polyporus dryadeus* springing from the rotted roots. In no instance were trees which were attacked by this fungus found in groups or even adjacent to each other The majority of the trees with this disease in their roots were growing under unfavorable environments. The boles of some of them were also attacked by various heart-rotting fungi, while others were perfectly sound above the collar, although they bore sporophores of *P. dryadeus* at the ground line.

No rhizomorphs of any kind were found associated with this rot, either beneath the bark, on the surface of the roots, or ramifying in the adjacent soil. How the lower part of the smaller roots became infected is not known.

The identity of the fungus causing this root-rot with the European fungus known as *Polyporus dryadeus* may be questioned. Through the courtesy of the officials in charge, the writer was permitted to examine all the American and European specimens of *P. dryadeus* in the following herbaria:

Pathological and Mycological Collections of the Department of Agriculture, at Washington, D. C., Herbarium of the New York Botanical Garden, and the Cryptogamic Herbarium of Harvard University.

Authentic specimens of *Polyporus dryadeus* from America, England, France, Germany, and Austria were examined, and a careful comparison of each with the material used as the basis of this article showed that the American plant under discussion is undoubtedly identical with the European fungus known as *P. dryadeus*.

There are three collections in the laboratory of the Office of Investigations in Forest Pathology, at Washington, D. C., of a Polyporus on *Tsuga heterophylla* from three widely separated localities in the State of Washington. These specimens were collected by C. J. Humphrey, of this office, and the legends accompanying them indicate that the sporophores were attached to the host at or near the surface of the ground and that the plant is a true parasite that kills the trees it attacks. These specimens agree in all essential characters, both gross and microscopic, with *Polyporus dryadeus*, and although the writer has not seen the rot produced in this host, he believes the fungus is this plant.

SUMMARY

(1) *Polyporus dryadeus* is a root parasite of the oak, producing a white sap rot and a heart rot in the roots.

(2) In all the trees examined this rot did not extend upward into the tree as a true heart or sap rot of the trunk, but was limited to the underground parts of the tree.

(3) The rot and sporophore described and figured by Robert Hartig do not belong to *Polyporus dryadeus*, but to *Polyporus dryophilus*.

(4) In the majority of cases only old or much suppressed trees or trees growing under very unfavorable conditions were found attacked by this disease.

(5) The disease does not seem to spread readily to adjacent trees.

(6) The disease is widely distributed both in America and in Europe and is probably found in these countries throughout the range of the oak.

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DESCRIPTION OF PLATES

PLATE XXI. Fig. 1.—*Polyporus dryophilus:* A median-longitudinal section of a sporophore on *Quercus alba* from Arkansas, showing the granular core and the white mycelial lines in the central and rear portion.

Fig. 2.—*Polyporus dryophilus:* Side view of the ungulate type of sporophore on *Quercus californica* from California.

Fig. 3.—*Polyporus dryophilus:* Median-longitudinal section of the globose type of sporophore on *Quercus garryana* from California, showing the large granular core and prominent white mycelial lines.

Fig. 4.—*Polyporus dryadeus:* Median-longitudinal view of a young sporophore on *Quercus texana* from Texas, showing the fibrous, non-granular nature of the context.

Fig. 5.—*Polyporus fulvus* Fries: Median-longitudinal view of a sporophore on *Quercus* sp. from Sweden, showing the granular core characteristic of *P. dryophilus*.

Fig. 6.—*Polyporus vulpinus:* Median-longitudinal view of sporophore on *Populus* sp. from Sweden, showing the granular core characteristic of *P. dryophilus*.

Fig. 7.—*Polyporus dryophilus:* Front view of the applanate type of a sporophore on *Populus tremuloides* from Colorado, showing the faint zones on the pileus where the hairs have disappeared.

Fig. 8.—*Polyporus dryophilus:* Median-longitudinal view of sporophore on *Populus tremuloides* from Colorado, showing the granular core originating between the sapwood and bark and extending into the center of the sporophore.

XXII. Fig. 1.—*Polyporus dryophilus:* Radial-longitudinal view of the rot occurring in *Quercus* sp. from Europe and said to be the rot produced by *P. dryadeus*.

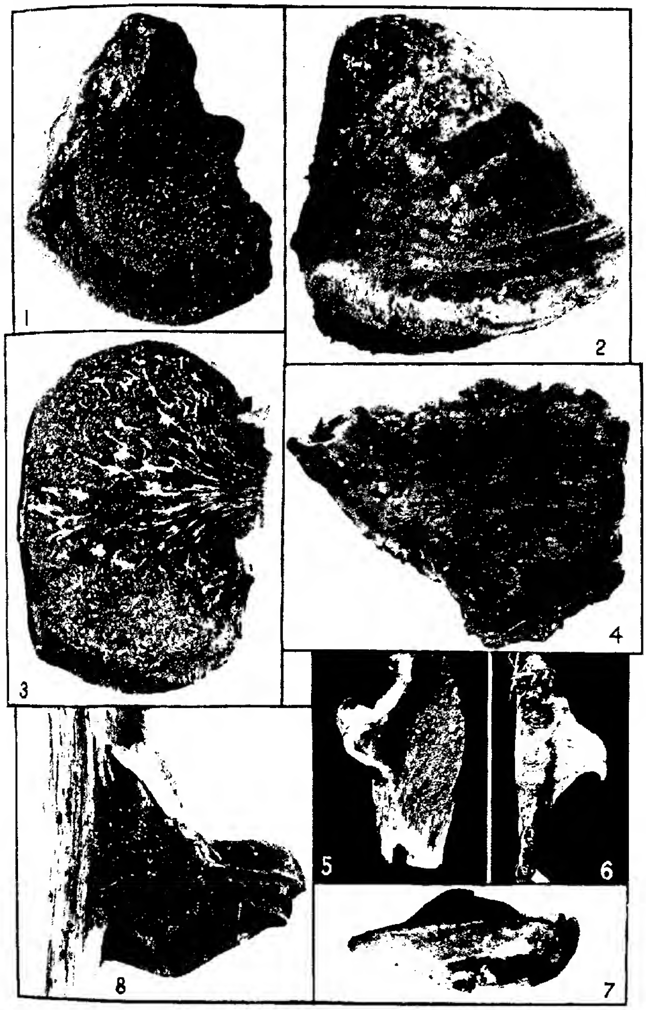
Fig. 2.—*Polyporus dryadeus:* Cross section of a small root of *Quercus alba* from Maryland, showing the mottled appearance of the diseased wood in the middle stages of the rot.

Fig. 3.—*Polyporus dryophilus:* Radial-longitudinal view of the rot appearing in *Quercus alba* from Arkansas, showing the advancing line of rot in a branch.

Fig. 4.—*Polyporus dryadeus:* Upper surface of a sporophore on roots of *Quercus texana* from Texas, showing the rough tuberculate pileus.

Fig. 5.—*Polyporus dryadeus:* Rot occurring in an apparently sound root of *Quercus alba* from Virginia, showing cross section of a diseased root, immediately adjacent to the point of attachment of a large sporophore of *P. dryadeus*, 1 foot high and 1 foot wide. Some sound, living wood is still present.

Fig. 6.—*Polyporus dryadeus:* Cross section of diseased root of *Quercus alba* from Virginia, showing the nearly sound, living upper half of the root and the badly diseased lower half.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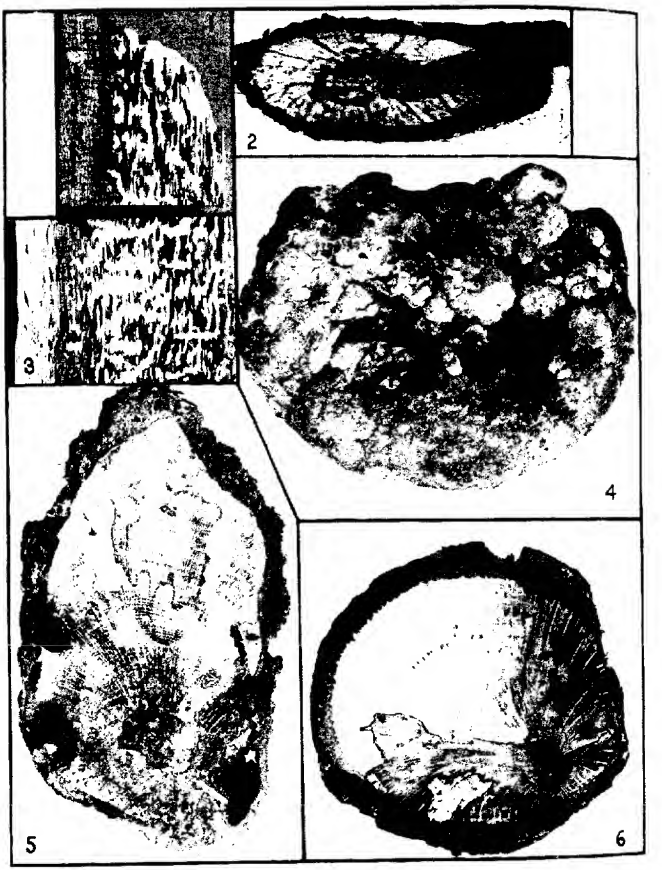
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THE FOOT-ROT OF THE SWEET POTATO

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INTRODUCTION

On August 9, 1912, Mr. O. H. Weiss sent the writer some diseased sweet-potato (*Ipomoea batatas*) vines from the vicinity of the Dismal Swamp, Va., with a request for information regarding the nature of the trouble. The stems for a short distance above the ground were covered with black fruiting bodies of a fungus, and suggested macroscopically the conidial stage of *Diaporthe batatatis*, the cause of the sweet-potato dry-rot. Careful examination of the material showed that in structure these fruiting bodies differed from those of the dry-rot organism, although it was apparent that both fungi belonged to the same general group. The organism was isolated in pure cultures from material taken from diseased sweet-potato stems and its parasitic habits and growth in artificial cultures compared with the dry-rot organism.

On August 22, 1912, the writer visited the sweet-potato fields near the Dismal Swamp in order to observe the disease under natural conditions and to ascertain the extent of the loss. The disease was found in practically every field, causing a loss of from 10 to 50 per cent of the crop, and in exceptional cases even more.

During August, 1913, the disease was found for the first time in many fields near Cape Charles and Keller, Va. Whether this is the first appearance of the disease in this part of the State is not known. The writer had inspected many fields in this section for several summers previous to 1913 and never observed the disease. It seems likely, therefore, that the disease is either new to these places or has heretofore occurred only to a very limited extent. The organism was isolated from specimens collected at both Cape Charles and Keller, and it was found to be identical with the one obtained during 1912 and 1913 from the vicinity of the Dismal Swamp.

Inquiry among the farmers in the vicinity failed to give a definite idea as to how long the disease has been prevalent. It was learned, however, that the disease has increased in severity in the last few years, and if not checked is likely to prove a serious handicap to the growing of a crop that would otherwise be a profitable industry.

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DIAGNOSIS OF FOOT-ROT

The foot-rot organism is a slow-growing parasite, especially during the earlier stages of infection. During about the first three weeks after inoculation, only a slight enlargement of the wound in all directions takes place. About three weeks seems to be required for the fungus to over come the plant sufficiently to cause any marked reduction in its vitality or vigor. As soon, however, as the fungus gets the upper hand, it develops very rapidly and in about one week more completely girdles and extends along the stem from 2 to 5 inches, killing the plant by the destruction of the cortex. At the end of about another week wilting of the leaves is first observed, the plants beginning to die soon afterwards. There is considerable variation in the length of time a plant will live after becoming infected, especially under greenhouse conditions, some of the plants dying in three or four weeks, while others may survive for one to four weeks longer. It is also interesting to note in this connection that those plants appearing to be the strongest when inoculated are likely to be the first to succumb to the disease. An explanation of this may be that a vigorously growing plant may stimulate the fungus to more rapid development.

The first sign of the disease of inoculated plants is a blackening of the cortex of the stem at the point of inoculation. When inoculated at the soil line, the fungus seldom grows more than half an inch below the surface of the ground, but it extends up the stem several inches. The leaves near the point of inoculation are invaded and soon turn yellow and fall off. Under greenhouse conditions numerous black pycnidia break through the epidermis of the stem (Pl. XXIII, fig. *A*) along the blackened area about the time the foliage begins to wilt. Under natural conditions in the field, on the other hand, the pycnidia form on the invaded tissue before the wilting of the plant. It was observed also that diseased plants will survive under field conditions much longer than in pots in the greenhouse, where they are naturally handicapped by artificial conditions. Many diseased plants in the field with fruiting bodies abundantly formed on the stem are often sustained by the roots which are thrown out at the nodes along the stems, although the main stem may be nearly destroyed by the fungus. If not supported by roots at the nodes, the diseased plants readily succumb.

As a rule, the disease is confined to the stem of the plant from the soil line to 4 or 5 inches above it. However, at Cape Charles, Va., in some of the low, rather wet fields, where there was a rank vegetative growth, vines were found diseased several feet from the hill. In such cases infection evidently took place at the node and spread in each direction (Pl. XXIV), the vine on each side of the diseased area remaining healthy. The organism isolated from pycnidia on such diseased spots was identical with the one obtained from the stem.

CAUSE OF THE FOOT-ROT

The organism causing the foot-rot of the sweet potato has been described as *Plenodomus destruens*.[1] It has also been pointed out that the fungus does not fit well into this genus or into any of the present-known genera. At the time, however, it was thought better to describe it as a new species of the genus Plenodomus rather than to create a new genus in a group where there are already a great many genera. It is probable that this organism is the conidial stage of an ascomycete which will eventually be discovered, and in view of that fact its generic position can only be temporary. It falls naturally in the order Sphaeropsidales and is more closely related to Phoma, Phomopsis, and Phyllostycta than to any of the other genera in the order.

The diagnosis of the genus Plenodomus as found in Saccardo's Sylloge Fungorum is somewhat brief. In 1911 Diedicke[2] worked over this genus, describing it more fully and pointing out the characteristics which distinguish it from Phomopsis, the genus with which it is most likely to be confused.

Since it is quite evident that the foot-rot fungus is not a Phoma, differing from that genus (1) in having more irregularly shaped pycnidia (Pl. XXV, *B*) and (2) in having a well-defined beak (Pl. XXV, *A*), attention will be given only to the characteristics which distinguish the foot-rot organism from Phomopsis, the conidial stage of the sweet-potato dry-rot.

According to Diedicke, Plenodomus is characterized by having only two walls composing the pycnidium—a dark outer wall and a hyaline one within. The outer wall completely surrounds the pycnidium and is of uniform thickness at the top and base. The inner hyaline layer is composed of several layers of cells and is somewhat thicker than the outer wall. The conidiophores are short, fragile, and inconspicuous. The spores are rounded at both ends.[3]

On the other hand, the pycnidium of Phomopsis, according to the same author, is composed of four walls. The upper portion of the pycnidium, especially about the beak, is composed of thick black cells. The dark color of this layer of cells becomes less conspicuous in the lower portion and practically disappears at the base of the pycnidium. Phomopsis is further characterized by the development of a stroma and chambering of the pycnidium. The conidiophores are long, conspicuous, and awl-shaped, and the spores are spindle-shaped. Because of the variation in the shape of the spores this latter character is of less importance than some of the others in separating the genus from Plenodomus. Stylospores are found in some species of Phomopsis.

[1] Harter, L. L. Foot rot, a new disease of the sweet potato. Phytopathology, v. 3, no. 4, p. 245–245, 1 fig., 1913.

[2] Diedicke, H. Die Gattung Plenodomus Preuss. Ann. Mycol., Jahrg. 9, No. 2, p. 137–141, pl. 8, 1911.

[3] This last character is perhaps of the least importance, since it is well known that the spores vary greatly within the genus and even in the same species. In fact, the spores of some species of Phomopsis have rounded ends.

It will be seen, therefore, that the following characteristics belonging to the dry-rot fungus are not found in the foot-rot organism: (1) Stroma; (2) chambering of the pycnidium; (3) conidiophores conspicuously long and awl-shaped; and (4) long, filiform, hook-shaped stylospores.

What is believed to be even more significant than the differences in morphological characters between these two organisms is the difference in parasitic habits and growth in artificial cultures. It has been pointed out in a previous bulletin[1] that the dry-rot fungus does not kill the plant but lives in apparent harmony with it without injury. The pycnidia appear on the stem only after the plant has been lifted and kept in a damp chamber for 10 days or 2 weeks, this being the first evidence that the plant was infected. The organism occurs on the petioles and leaves of dead plants and often develops on apparently sound roots after a period of time in storage. Stylospores are frequently found on the roots and stems.

The foot-rot disease, on the other hand, kills the plant in three to eight weeks after infection by the destruction of the cortex of the stem for several inches above and a little distance below the surface of the soil. Pycnidia are formed on the diseased portion of the stem about the time the foliage begins to wilt (Pl. XXVI, fig. *A*), and under field conditions even earlier.

The growth of the organism on several kinds of the commonly used artificial media and especially on synthetic agar[2] and on corn meal[3] furnishes additional means of distinguishing the two diseases.

On synthetic agar the foot-rot fungus grows slowly and under normal conditions forms a very compact growth, at first irregular in outline with a slightly darker center, attaining a diameter of not more than 2 or 3 mm. at the end of a week or 10 days. (Pl. XXVII, fig. *B*.) On the same culture medium the dry-rot fungus grows much faster, forming a loose, flaky growth of uniformly white hyphæ having an irregular outline. (See Pl. XXVII, fig. *A*.) The growth of the dry-rot fungus is so loose and inconspicuous that it is scarcely visible until it has attained a diameter of 2 or 3 mm.

[1] Harter, L. L., and Field, Ethel C. A dry rot of sweet potatoes caused by Diaporthe batatatis. U. S. Dept. of Agr., Bur. Plant Indus., Bul. 281, 38 p., 4 pl., 1913.

[2] Synthetic agar is prepared as follows:

	Grams.
Distilled water	1,000
Dextrose	200
Peptone (Witte's)	10
Ammonium nitrate	10
Potassium nitrate	5
Magnesium sulphate	2.5
Calcium chlorid	0.1
Agar agar	20

Place the water in the beaker first; then add other ingredients in the order given. Stir and let stand till the agar agar is moist. Steam 1 hour. Tube with constant stirring. Plug and autoclave for 15 minutes at 110° C. Agar of high purity only should be used.

[3] Corn-meal flasks are prepared as follows: Place 5 grams of corn meal in a 100 c. c. flask. Add 45 c. c. of distilled water and steam for 15 minutes. Plug and autoclave at 11 pounds pressure for 20 minutes.

On corn meal the dry-rot organism forms a black stroma composed of several pycnidia with long exserted beaks. The stroma is ½ to 1 or more mm. in diameter and is preceded by a profuse growth of mycelia. The foot-rot organism, on the other hand, forms no stroma on corn meal. The pycnidia stand separately and are very numerous, while the mycelial growth is slight and inconspicuous. The pycnidia follow closely after the growth of hyphæ, the pycnidial zone increasing with the increase in diameter of the mycelial growth. Spores are exuded in great quantities, forming a yellowish transparent liquid over the surface of the medium.

ISOLATION OF THE FUNGUS

Pure cultures of the foot-rot organism were particularly easy to secure by the poured-plate method. Stems on which the pycnidia were present were thoroughly washed in hydrant water or, preferably, disinfected with mercuric chlorid for about 40 seconds and then rinsed in sterile water. A few of the pycnidia were then macerated in a watch glass in sterile water and one or two loopfuls transferred to tubes of synthetic agar and plates poured. The fungus grows very slowly on agars, particularly on synthetic agar. The colonies are not visible in the plates for three days and often not until five or six days after they are made. Because of the characteristic growth on synthetic agar the organism can easily be picked out from other fungi when the appearance of the colony is once known.

DESCRIPTION OF THE FUNGUS

MYCELIUM.—The appearance of the mycelium varies so markedly on different culture media and according to the age of the culture that it would be difficult to give a simple, characteristic, general description. In young cultures and for the most part in old cultures it is nearly always hyaline, although occasionally browned hyphæ may be found. Oil globules are found in the mycelia at all ages (Pl. XXV, *C*). Hyaline, spherical and oval, thick-walled bodies 8 to 13μ in diameter, generally filled with oil globules, intercalated or, rarely, terminally, in chains or singly (Pl. XXV, *D*), occur in most media and at nearly all ages. Browned bodies morphologically similar to the hyaline ones but occurring mostly at the end of the hyphæ (Pl. XXV, *E*) are frequently found in older cultures. In 7-months-old corn-meal cultures which were quite well dried out the brown bodies were abundant, especially where the media came in contact with the glass. In these cultures the hyaline forms were few. In 4-months-old cultures of string beans brown and hyaline bodies and brown hyphæ were present. The brown hyphæ were filled with numerous beadlike swellings. On the other hand, in a rice culture of the same age only hyaline hyphæ and hyaline spherical or oval bodies were found.

PYCNIDIA.—The pycnidia are at first buried, but later break through the epidermis, appearing as black dots scattered over the surface. They stand close together on the stem and roots, but they are not confluent

or only rarely so. (Pl. XXIII, fig. *A*.) They are irregular in form and vary greatly in size, averaging about 300μ through their greatest diameter.

In cross section the pycnidia from the stem and roots show somewhat different structures. From either source they are completely inclosed by a dark, almost black, outer wall (Pl. XXV, *A* and *B*).

The pycnidia on the roots have a well-defined inner hyaline layer almost equal in thickness to the outer wall (Pl. XXV, *A*). On the stem the dark wall is more conspicuous, being better developed than on the root, and the inner hyaline layer is completely lacking (Pl. XXV, *B*).

The basidia are short, fragile, somewhat inconspicuous, and arise from the inner hyaline layer or from the dark wall in pycnidia where the hyaline layer is absent. They are 6 to 13μ in length and very narrow.

The spores are discharged through a beak varying somewhat in length, which may arise from any part of the upper surface of the pycnidium. In old dried specimens the upper portion of the pycnidium may fall away.

PYCNOSPORES.—The pycnospores are oblong, rounded at both ends, 6.8 to 10.0μ long by 3.4 to 4.1μ wide, with two large oil droplets. They are hyaline, 1-celled, and sometimes slightly curved (Pl. XXV, *F*).

In the same pycnidium on the host and occasionally on rice and on sweet-potato-stem cultures are found in addition to the pycnospores hyaline curved or straight bodies 6 to 15μ in length. These bodies are somewhat cylindrical in shape and rounded or tapering at the ends (Pl. XXV, *G*). The function of these bodies is not known. Several attempts have been made to germinate them, and while there have been some reasons to believe that a germ tube was developed, this point was not definitely settled. These bodies were formed so sparingly in artificial media that it was necessary to use those from the host in order to test their germination in hanging-drop cultures in Van Tieghem cells. Because of the difficulty in sterilizing this material, bacteria completely overran the cultures in about 24 hours, thus terminating the experiment.

PARASITISM OF THE ORGANISM

INOCULATION EXPERIMENTS

The details of inoculations with Plenodomus are found in the following pages. For convenience, the experiments are numbered and arranged according to dates of inoculation and under the heading to which they belong. The organisms used to make the inoculations are also designated by numbers.[1]

[1] For convenience and ready reference, separate numbers (100, 101, 102, 108, and 110) were given to the different isolations where they or subcultures from them were used for inoculations. No. 100 was given the organism obtained from specimens sent the writer Aug. 9, 1912, and No. 101 from specimens collected Aug. 22 from the same locality. The other numbers used, 102, 108, and 110, were given to the organism reisolated from inoculated plants. A new number was given the fungus only when it was the source from which other plants were to be inoculated. However, it should be kept in mind that these different numbers represent only different isolations of the same organism (*Plenodomus destruens*).

Most of the inoculations were made in the greenhouse, principally because they were performed in the winter. One set, however, which was conducted in the field, gave results so similar to those in the greenhouse that it was not possible to distinguish between them in any essential details. The plants for inoculation were obtained from sound potatoes carefully selected for the purpose. They were grown in pots of sterilized soil and kept far enough apart to prevent accidental infection from watering and overlapping of the vines. Only strong, vigorously growing plants were inoculated, all others being thrown out. That there was probably no accidental infection is shown by the fact that not a single check in the whole series of inoculations became diseased.

Inoculations in the Field

Experiment No. 1.—On August 26, 10 sweet-potato plants, the vines being about 3 feet long, were inoculated [1] on the Potomac Flats near Washington, D. C., by inserting pycnospores and hyphæ of organism No. 102 (culture No. 1 of Aug. 15) into the lower part of the stem. Ten plants pricked with a sterile needle were used as checks.

Results.—On September 18 all the inoculated plants were infected,[2] the plants turning yellow, and the lower leaves dropping off. The periphery of the stem for 3 to 5 inches above the ground was black, and pycnidia were abundantly formed thereon. The stems were blackened throughout, but attempts to isolate the fungus from the fibro-vascular bundles gave negative results. None of the checks were diseased. The infected plants were all lifted on October 10, taken to the laboratory, and examined. Pycnidia were present on all. On October 12 cultures were made from seven of these plants, and the organism recovered [3] in each case.

Inoculations in the Greenhouse

Experiment No. 2.—On August 26, 1912, 10 young sweet-potato plants in pots were inoculated with organism No. 100 (culture No. 8 of Aug. 15) by inserting pycnospores and hyphæ into the stem at the soil line. Five plants pricked with a sterile needle were left as checks.

Results.—On September 16 four plants, on November 14 one, and on November 25 three, or a total of eight plants, were infected. None of the checks were diseased. Pycnidia were formed on all the diseased plants and the organism recovered from three. The experiment was terminated December 2, 1912.

Experiment No. 3.—On November 13 ten young sweet-potato plants in pots were inoculated as in experiment No. 2 with organism No. 101 (culture No. 9 of Oct. 31). Six plants pricked with a sterile needle were left as checks.

Results.—On December 14 one plant, on December 18 three, on December 21 one, on December 26 one, on December 30 two, and on January 10 two, or a total of ten plants, were infected. Pycnidia were present on eight plants when lifted and developed on the other two after two days in a moist chamber. All the checks remained healthy. The experiment was terminated January 17, 1913.

[1] All inoculations recorded in this article, unless otherwise stated, have been made from cultures grown on sterile moistened corn meal and only when spores were exuding from the pycnidia.

[2] By "infected" is to be understood the stage when the plant began to wilt and die. It was generally quite evident some days earlier that the plants were infected, although they were not so recorded until this stage was reached.

[3] No attempt has been made to recover the organism from all diseased plants. Occasionally, however, the fungus was recovered from infected plants in order to compare it with the original strain, or for the purpose of inoculating it into other plants.

EXPERIMENTS NOS. 4, 5, 6, AND 7.—On November 18 four sets of inoculations were made of 10 plants each (40 plants in all) with organism No. 101 (culture No. 8 of Oct. 31), as follows: (No. 4) By smearing pycnospores on the leaves and spraying the foliage with spores suspended in sterile water and covering the plants with bell jars for 24 hours, (No. 5) by smearing pycnospores on the base of the stem, (No. 6) by pouring pycnospores suspended in sterile water about the plants, and (No. 7) by inserting pycnospores and hyphæ into the base of the stem. Six plants were left as checks.

Results.—(No. 4) No infection. (No. 5) On December 30 one plant, on January 8 one, on January 13 two, on January 15 one, on January 23 one, and on January 30 one, or a total of seven plants, were infected. Pycnidia were abundant on all when lifted. (No. 6) On December 26 one plant, on December 30 one, on January 4 two, on January 6 one, on January 10 one, and on January 13 one, or a total of seven plants, were infected. The infected plants were lifted on January 24 and pycnidia were present on all. (No. 7) On December 21 two plants, on December 26 one, on December 28 two, on January 6 one, on January 11 one, on January 13 one, on January 14 one, and on January 17 one, a total of ten plants, or all of those inoculated, were infected. Pycnidia were present on nine of these plants when lifted and developed on the other one after three days in a moist chamber. None of the checks were diseased. The experiment was terminated on February 27.

EXPERIMENT No. 8.—On December 9 six 5-weeks-old sweet-potato plants in pots were sprayed with pycnospores and hyphæ of organism No. 101 (culture No. 1 of Nov. 12) suspended in sterile water. The plants were covered with bell jars and shaded with paper for 24 hours. Six plants were left as checks.

Results.—No infection. The experiment was terminated February 27, 1913.

EXPERIMENT No. 9.—On December 28 eight 4-months-old sweet-potato plants grown in pots were inoculated by inserting pycnospores and hyphæ of organism No. 100 (culture No. 2 of Dec. 10) into the base of the stem. Six plants pricked with a sterile needle were left as checks.

Results.—On January 23 one plant, on February 4 one, on February 7 three, and on March 8 one, or a total of six plants, were infected. The checks remained healthy. Pycnidia were present on all the infected plants when lifted. The organism was recovered from two of the infected plants. The experiment was terminated March 27.

Only young plants were used in the first eight experiments. Experiment No. 9 was made with old plants (as compared with those used in experiment No. 8) for the purpose of determining whether they were as susceptible as young ones to the foot rot. The results indicate that they are.

EXPERIMENT No. 10.—On January 23, 1913, six sweet-potato plants (three old and three young) grown in pots were sprayed with pycnospores of organism No. 100 (culture No. 3 of Dec. 28) suspended in sterile water. All the plants were making a good growth. As soon as the plants were sprayed, they were covered with bell jars and manila paper for 48 hours. Six plants were left as checks.

Results.—None of the plants were infected. The experiment was terminated March 27, 1913.

EXPERIMENTS NOS. 11 and 12.—On January 17 ten young plants, each of *Ipomœa purpurea* (L.) Roth. and *Ipomœa hederacea* Jacq. were inoculated with organism No. 100 (culture No. 4 of Dec. 28). Seven plants were left as checks.

Results.—No infection.

EXPERIMENT No. 13.—On December 2 five young plants of *Ipomœa coccinea L.* in pots were inoculated at the base of the stem with organism No. 101 (culture No. 2 of Nov. 12). Five plants were left as checks.

Results.—On February 28, 1913, three plants were infected. The organism from two of the plants was recovered by pouring plates from the pycnidia and from the third plant by planting bits of diseased tissue in plates of synthetic agar.

None of the checks became diseased. The experiment was terminated February 28, 1913.

EXPERIMENT No. 14.—On May 9 seven sweet-potato plants in pots in the greenhouse were inoculated by inserting the hyphæ (no pycnidia in the culture) of organism No. 101 (culture No. 2 of May 5) into the lower part of the stem. Six plants were left as checks.

Results.—On May 31 six plants, and on June 4 one, or a total of seven plants, were infected. None of the checks were diseased. When the experiment was terminated on June 5, pycnidia were abundant on the stems of all diseased plants.

EXPERIMENT No. 15.—On September 3 six sweet-potato plants in pots in the greenhouse were inoculated by inserting spores and hyphæ of organism No. 101 (culture No. 13 of Aug. 14) into the vine at the node 3 to 4 feet from the hill. Five other vines were wounded with a sterile needle and left as checks.

Results.—On September 25 five of the vines were infected at the point of inoculation. The organism had spread 2 inches or more each way from the point of inoculation. None of the checks were diseased. The experiment was terminated October 5, 1913.

EXPERIMENT No. 16.—On September 3 five sweet-potato plants in pots in the greenhouse were inoculated by inserting spores and hyphæ of organism No. 108 (culture No. 17 of Aug. 18) into a vine at the node 3 to 4 feet from the hill. The checks were the same as those used in experiment No. 15.

Results.—On September 25 all the vines were infected at the point of inoculation, the organism spreading as in experiment No. 15. The experiment was terminated October 5, 1913.

INOCULATIONS FROM REISOLATIONS

EXPERIMENT No. 17.—On October 5 twelve young sweet-potato plants in pots were inoculated by inserting pycnospores and hyphæ of organism No. 102[1] (culture No. 2 of Sept. 25) into the lower part of the stem. Ten plants pricked with a sterile needle were left as checks.

Results.—On November 5 five plants, on November 11 one, on November 13 one, on November 15 one, on November 25 one, and on December 9 three, or a total of all 12 plants, were infected. None of the checks were infected. Pycnidia were found on ten of these plants when lifted and developed on the other two after three days in a moist chamber. The organism was recovered in pure cultures from seven plants. The experiment was terminated on December 9, 1913.

EXPERIMENT No. 18.—On January 23 eight young sweet-potato plants in pots were inoculated by inserting pycnospores and hyphæ of organism No. 108[2] (culture No. 2 of Jan. 11) into the lower part of the stem. Six plants were left as checks.

Results.—On February 28 one plant, on March 8 two, on March 13 four, and on March 28 one, or a total of eight plants, were infected. Pycnidia were present on seven of the diseased plants when lifted and developed on the other one after 10 days in a moist chamber. None of the checks were diseased. Experiment terminated March 29, 1913.

EXPERIMENT No. 19.—On February 19 ten young sweet-potato plants in pots were inoculated by inserting pycnospores and hyphæ of organism No. 108 (culture No. 3 of Jan. 11) into the stem. Seven plants were left as checks.

Results.—On March 21 four plants, on March 24 one, on March 31 one, on April 4 one, on April 18 one, and on April 26 one, or a total of nine plants, were infected.

[1] The organism recovered from plants inoculated in the greenhouse on Aug. 26 with No. 100 is known as No. 102.

[2] When the plants, inoculated on the Potomac Flats on Aug. 26, 1912, were dug, they were placed with the roots attached in moist chambers in the laboratory. After several weeks the fungus grew from the stem into the roots (Pl. XXIII, *B*), from which it was recovered. This organism was numbered "108."

Pycnidia were abundant on eight of the diseased plants when lifted on April 22. The one remaining diseased plant was lifted on April 26, and pycnidia were then present. The experiment was terminated April 26, 1913.

EXPERIMENT No. 20.—On March 13 six young sweet-potato plants were inoculated by inserting spores and hyphæ into the lower part of the stem with organism No. 110[1] (culture No. 3 of Mar. 5). Five plants were left as checks.

Results.—On April 18 two plants, on April 22 two, and on April 23 one, or a total of five plants, were infected. The diseased plants were lifted on April 26, and pycnidia were present on all. None of the check plants were diseased. The experiment was terminated April 26, 1913.

INOCULATIONS IN THE LABORATORY

EXPERIMENT No. 21.—On November 18 eight mature sweet potatoes (not plants) were inoculated by inserting pycnospores and hyphæ of organism No. 101 (culture No. 8 of Oct. 31) into the end of the potatoes. They were placed in cloth bags and stored in the laboratory. Four potatoes pricked with a sterile needle were used as checks.

Results.—No infection. The experiment was terminated January 31, 1913.

EXPERIMENT No. 22.—On April 4, 1913, six sound sweet potatoes were prepared for inoculation by cutting away the ends of each so as to leave nothing but healthy tissue. They were then thoroughly washed and disinfected by treating with mercuric chlorid (1:1,000) for five minutes. They were afterwards rinsed in sterile water and placed in a moist chamber on filter paper disinfected with corrosive sublimate. Three of the potatoes were inoculated at the end and three at the side by inserting spores and hyphæ of organism No. 108 (culture No. 1 of Mar. 8). Four other potatoes pricked with a sterile needle were used as checks.

Results.—On April 15 no signs of decay had started at the point of inoculation. The filter paper appeared a little dry, and sterile water was added. After April 15 the rot developed and progressed rapidly in all the potatoes from the point of inoculation until by May 1 one potato was completely decayed and the others about one-third. Plate XXVIII, figure *A*, shows a sweet potato inoculated at the end and figure *C*, one inoculated at the side. Figures *B* and *D* are sections of figures *A* and *C*, respectively, showing the extent of the rot. The potatoes inoculated at the side decayed more rapidly than those inoculated at the end. Mature pycnidia and spores were formed on the surface on May 1. The organism was recovered from the pycnidia and from the diseased brown tissue of two potatoes.

The organism causes a chocolate-brown to almost black discoloration of the tissue, but leaves it rather firm, even in the later stages. This is not a distinctive characteristic, since there are a number of rots of the sweet potato, nearly all of which produce some shade of brown in the tissue and are in general so similar that it is practically impossible to separate them by their macroscopic appearances. All of the check potatoes remained sound.

[1] This organism was obtained from plants of *Ipomoea coccinea* which were inoculated with organism No. 101.

TABLE I.—*Summary of results of inoculations with Pl*

Organism No.	Host.	Place of inoculation.	Method of inoculation.	Number— Inoculated.	Infected.	Checks.	Number of checks infected.	Experiment No.[1]
100....	Ipomoea batatas.	Potomac Flats.	By inserting spores and hyphæ into the lower part of the stem.	10	10	10	0	1
100....	....do.....	Greenhouse.	.....do.....	10	8	5	0	2
101....	....do.....	....do.....	.....do.....	10	10	6	0	3
101....	....do.....	....do.....	.....do.....	10	10	6	0	7
100....	....do.....	....do.....	.....do.....	8	6	6	0	9
100....	Ipomoea purpurea.	....do.....	.....do.....	10	0	7	0	11
100....	Ipomoea hederacea.	....do.....	.....do.....	10	0	7	0	12
101....	Ipomoea coccinea.	....do.....	.....do.....	5	3	5	0	13
102....	....do.....	....do.....	.....do.....	12	12	10	0	17
108....	....do.....	....do.....	.....do.....	8	8	6	0	18
108....	....do.....	....do.....	.....do.....	10	9	7	0	19
110....	....do.....	....do.....	.....do.....	6	5	5	0	20
101....	....do.....	....do.....	By spraying foliage with spores suspended in water.	10	0	6	0	[2] 4
101....	....do.....	....do.....	.....do.....	6	0	6	0	8
100....	....do.....	....do.....	.....do.....	6	0	6	0	10
101....	....do.....	....do.....	By smearing conidia on lower part of stem.	10	7	6	0	5
101....	....do.....	....do.....	By pouring spores in water around the plant.	10	7	6	0	6
101....	Ipomoea batatas.	....do.....	By inserting hyphæ into the lower part of stem.	7	7	6	0	14
101....	....do.....	....do.....	By inserting spores and hyphæ into the node of vine several feet from the hill.	6	6	5	0	15
108	....do	....do.....	.....do.....	5	5	5	0	16
101....	Storage sweet potatoes.	Laboratory.	By inserting spores and hyphæ into the end of potato.	8	0	4	0	21
108....	....do.....	....do.....	.....do.....	6	6	4	0	22

[1] For more complete data, the reader is referred to the experiments in the preceding pages corresponding to the numbers of this column.
[2] Experiments Nos. 4 to 7, inclusive, are combined in the body of the text, p. 258.

DISCUSSION OF INOCULATION EXPERIMENTS

Twenty-two sets of inoculations have been made with *Plenodomus destruens*, 17 of which were on sweet-potato plants. Eighty-four sweet-potato plants in nine different sets were inoculated by wounding the lower part of the stem and inserting spores and hyphæ. Seventy-eight died of the disease. Seven plants were wounded in a similar manner and inoculated with hyphæ only, and all became infected. Eleven vines in two sets were inoculated at the node several feet from the hill and 18 became diseased. Spores and hyphæ were smeared on the lower part of the stems of 10 plants, care being taken to cause no wounds, and 7 became diseased. Spores suspended in sterile water were poured about 10 plants, and 7 died from the organism. The foliage of 26 plants in

three different sets was sprayed with the spores suspended in water, but the disease was not produced thereby. Ten plants each of *Ipomoea hederacea* and *Ipomoea purpurea*, and 5 plants of *Ipomoea coccinea* were inoculated by inserting spores and hyphæ into the lower part of the stem. Three plants of *Ipomoea coccinea* were infected, the other species not being injured by the fungus.

Two sets of inoculations have been made with potatoes taken from storage. After inoculation one set was kept in the laboratory room in a cloth bag and gave negative results. In the other experiment the potatoes were placed in a damp chamber and kept moist with filter-paper saturated with mercuric chlorid. Under these conditions the potatoes rotted readily. (Pl. XXVIII, figs. *A*, *B*, *C*, and *D*.) The organism was recovered in pure culture from the pycnidia formed thereon and from the rotten tissue within.

The results of these experiments show that the foot-rot organism is a vigorous wound parasite of *Ipomoea batatas*. In the greenhouse and in the field infection can be readily produced by wounding the plant, but this method is not imperative. It has been further shown that the temperatures and other environmental factors best suited for the growth of the plants are likewise most favorable for the development of the fungus. During warm, moist weather, when the plants grow most vigorously, the disease was more severe than when growth was retarded by low temperature. Plants at all ages were about equally susceptible to the disease.

It is also interesting to note in this connection that infection was readily produced by inoculating with hyphæ only, the result showing that the progress of the disease was more rapid and the plants killed sooner than when inoculations were made with spores.

HOW THE DISEASE IS PERPETUATED

The exact life history of this fungus will be in doubt so long as a perfect stage is not known. It is evident, however, that an ascogenous stage is not necessary to carry it from one season to the next. Diseased specimens on which there were numerous pycnidia were wintered out in a wire cage covered over with leaves and some dirt with the hope that an ascospore stage might develop. On the 27th of the following April the specimens were examined, and normal pycnospores but no asci were found.

A second lot of diseased specimens were wintered out in a wire cage set on the ledge of a north window, where they were subjected to alternately dry and wet weather and other atmospheric changes. When these were examined on May 20, 1913, numerous normal conidia were present and the organism recovered in culture.

There are at least two ways by means of which this disease may be carried from one year to another: (1) On the dead vines and (2) on the

potatoes in storage. In the locality in which this disease occurs, the hotbeds are started about April 1, or even sooner, so that infection of young plants might easily take place from pycnospores that had endured as late as May 20. In old fields the beds are often made from the soil on which sweet potatoes have been grown the previous year, thereby providing the best conditions possible for direct infection of the new crop. Furthermore, it was previously pointed out that the foot-rot organism spreads from diseased stems to the potatoes and develops pycnidia thereon. Experiments have also shown that under hotbed conditions the organism will grow from diseased potatoes on to the slips produced therefrom. Therefore, owing to the comparative obscurity of diseases of this type, infected roots might readily be overlooked when selecting seed, thereby making the sprouts growing from such potatoes liable to infection.

The brown, spherical, thick-walled, chlamydosporelike bodies were found in abundance embedded in the cortex of diseased parts of plants wintered out in the wire cages. What function these forms have is not yet known, although it is possible that they are able to reproduce the fungus and serve to carry the organism through unfavorable conditions. Repeated attempts, however, to germinate them have always given negative results.

SOME PHYSIOLOGICAL CHARACTERISTICS OF THE FUNGUS

CHARACTER OF GROWTH ON DIFFERENT CULTURE MEDIA

The foot-rot fungus grows well on some kinds of media, but sparsely on others. The growth on some media may be regarded as characteristic of the organism and is unlike that of any other fungus with which the writer is familiar.

A comparative study of growth has been made on nine different culture media—i. e., corn meal, string-bean agar, string beans, Irish-potato cylinders, sweet-potato cylinders, sweet-potato stems, rice, beef bouillon, and beef agar. These different media have not been selected for any particular reason, except that they are those commonly used and can easily be duplicated. Five tubes (flasks in case of corn meal) were inoculated on November 25, 1912, with conidia from a 25-day-old culture grown on corn meal. The tubes and flasks were kept in the light on a table in the laboratory, the temperature of which varied from 18° to 24° C. They were kept under observation until January 31, 1913, after which, owing to the dried condition of the cultures, no more notes were made. The following records, given in number of days from the beginning of the experiment, show the nature of the growth on the different media.

CORN MEAL (1061[1])

2 days.—No visible growth.

4 days.—Yellowish white growth about 1 cm. in diameter. Hyphæ growing close to the medium.

7 days.—Hyphal growth about 4 cm. in diameter, slightly yellowish. Numerous minute black pycnidia covering an area of about 2 cm. in diameter in the center of the growth.

9 days.—Hyphal growth covering most of the surface of the medium and pycnidia formed over about two-thirds. Spores just beginning to exude from pycnidia.

11 days.—Pycnidia covering most of surface of medium; exudate of spores forming small viscid droplets.

14 to 17 days.—Abundant discharge of spores from the pycnidia.

21 days.—Spore discharge collecting in large globules, forming an almost continuous covering over the surface of the medium.

25 days.—No change.

40 days.—Surface of medium completely covered with a slimy liquid containing pycnospores.

67 days.—Hyphæ hyaline. Numerous intercellular and terminal chlamydosporelike bodies.

Corn meal is the best of the media used for the development of pycnidia. The pycnospores are first expelled in about one week, the process continuing for 30 or 40 days thereafter. At the end of that time the medium is covered with a slimy liquid in which the spores are suspended. This liquid, often amounting to 5 c. c., is characteristic of growth on this medium and is apparently not due to the water added, since that is taken up by the corn meal.

STRING-BEAN AGAR (1037)

4 days.—Sparse white growth.

7 days.—Heavy, white flaky growth of erect hyphæ covering one-fourth of slant.

9 days.—Light-colored pycnidia collected in spots on surface of medium.

11 days.—No increase in mycelial growth; pycnidia dark; pycnospores exuding from pycnidia.

14 to 17 days.—Perceptible increase in the exudation of pycnospores.

21 days.—Exudate colorless, forming large droplets and uniting.

25 days.—No apparent change.

40 days.—Hyphal growth covering most of slant. Spores normal.

67 days.—Hyphæ hyaline. A few chlamydosporelike bodies.

String-bean agar is only a fair medium for the growth of this fungus. The pycnidia were sparingly formed as compared with the growth on corn meal.

STRING BEANS (1063)

4 days.—White, loose, flaky growth covering one-third of medium.

7 days.—White, loose, flaky growth covering three-fourths of medium.

9 days.—Felty grayish white growth of somewhat erect hyphæ. Pycnidia collected in spots. Pycnospores present.

11 days.—Pycnidia black.

14 days.—Slight exudate of spores from pycnidia.

17 days.—Slight increase in the discharge of spores.

21 to 25 days.—Exudates uniting, colorless.

[1] A number is given to and a description made of each medium when it is prepared in the laboratory so that it can be readily duplicated when desired. Unless otherwise stated, all media were prepared in the laboratory of the Office of Cotton and Truck Disease and Sugar-Plant Investigations.

40 days.—Medium studded with pycnidia. Exudate abundant. Pycnospores not typical, being immature in appearance and irregular in shape.

67 days.—Hyphæ hyaline. Many chlamydosporelike bodies. Long cylindrical bodies present. (Pl. XXV, *G*.)

IRISH-POTATO CYLINDERS (1036)

4 days.—Dense, felty white growth covering all of potato cylinder. Medium slightly darkened.

7 days.—Scattered dark (not black) pycnidia forming.

9 days.—Pycnidia abundant, irregularly scattered; black, rather large.

11 days.—Pycnidia black and conspicuous; uniformly scattered over the medium.

14 days.—A slight exudate of spores from pycnidia.

17 days.—Pycnidia crowded together. Slight discharge of spores.

21 to 25 days.—Pycnidia numerous. No discharge of spores from the pycnidia.

40 days.—Potato cylinder studded with pycnidia. No discharge of spores. Pycnospores abnormal, being apparently immature and irregular in shape.

67 days.—Hyphæ hyaline. A few chlamydosporelike bodies. Long cylindrical bodies present. (Pl. XXV, *G*.)

SWEET-POTATO CYLINDERS (1064)

4 days.—White procumbent growth of fairly dense hyphæ covering one-half of potato cylinder. Medium changed to a light chocolate-brown color.

7 days.—Feltlike growth covering all of medium. Potato cylinders changed to a chocolate-brown color. Pycnidia forming; surface of medium grayish.

9 days.—Pycnidia crowded together, forming a felty grayish surface on the medium.

11 days.—Pycnidia formed in a dense grayish mass over surface of medium. Spores exuding from the pycnidia.

14 to 25 days.—Slight discharge of spores.

40 days.—Medium covered with pycnidia. Spores exuding abundantly from one tube, a little from another, and none from the remaining tubes.

67 days.—Many long cylindrical bodies. Hyphæ hyaline.

SWEET-POTATO STEMS (1049)

4 days.—A sparse spreading growth of white hyphæ covering one-fourth of stem.

7 days.—Sparse, grayish, somewhat irregular, cottony growth of erect hyphæ. Pycnidia black, larger than on corn meal, and resembling those on the vines under natural conditions.

9 days.—Pycnidia black, uniformly distributed over medium. Spores exuding from the pycnidia.

11 days.—Pycnidia numerous; pycnospores exuding from pycnidia in brownish globules.

14 to 17 days.—Increase in the discharge of spores from the pycnidia.

21 to 25 days.—Exudates from the pycnidia uniting.

40 days.—Stems studded with pycnidia with long beaks. Discharge of spores from the pycnidia less than on corn meal.

67 days.—Hyphæ somewhat brown. A few long cylindrical bodies

RICE (967)

4 days.—No visible growth.

7 days.—Very slight mycelial growth. Many black, somewhat large pycnidia.

9 days.—Mycelial growth sparse. Spores just beginning to ooze from the pycnidia.

11 days.—Surface of medium studded with black pycnidia. Spores discharged from the pycnidia in small globules.

14 to 17 days.—Discharge of spores from the pycnidia increasing.

21 to 25 days.—Exudates from the pycnidia increasing and uniting.

40 days.—Pycnidia abundant, forming a black crust over the surface of the medium. Exudate of spores from the pycnidia a yellowish slimy mass.

67 days.—A few long cylindrical bodies present. Hyphæ mostly hyaline. No chlamydosporelike bodies.

Rice, with the exception of corn meal, is the best of all the media tried. Spores are formed abundantly and exuded in large droplets from the pycnidia. A very scant mycelial growth is formed on rice.

BOUILLON (5725[1])

3 days.—No visible growth.

6 days.—A very sparse flaky white growth arising from individual spores lodged against the glass below the surface of the medium.

8 days.—Growth below the surface of the medium from individual spores enlarging and adhering to the glass. No floating hyphæ. Slight surface growth against the glass.

10 days.—Increase in mycelial growth.

14 days.—Pycnidia forming a black ring against the glass at the surface of the medium.

29 days.—Pycnospores very few, poorly developed, and not typical of the spores on corn meal or rice.

56 days.—Hyphæ hyaline, with many intercellular, spherical swellings singly or in chains.

BEEF AGAR (5726[1])

3 days.—Grayish, thick, felty growth, extending ¼ cm. above surface of medium; irregular in outline.

6 days.—Growth spreading; white pycnidia forming.

8–10 days.—Pycnidia forming a black line on the surface of the medium at the point of contact with the glass; elsewhere on the surface of medium they are collected into spots.

14 days.—Pycnidia large and black and increasing in number.

29 days.—No pycnidia at the edge of mycelial growth except in contact with glass. Spores few and imperfectly formed.

56 days.—Hyphæ hyaline, with many intercellularly or terminal spherical bodies several times the diameter of the hyphæ arranged singly or in chains. Very few typical spores.

These results of tests with the different media[2] bring out clearly the fact that the development of the foot-rot organism is decidedly good on some media and very poor on others. Numerous pycnidia and an

[1] This medium was prepared in the Laboratory of Plant Pathology.

[2] In addition to the results of growth obtained on the nine media discussed in the preceding pages, the fungus was cultivated on a number of others, but not for the purpose of comparing the growth at the end of stated periods of time; hence, these have not been included in the general description. Growth of the fungus has been studied on mature stems of cotton (*Gossypium herbaceum*), sweet clover (*Melilotus alba*), also on immature stems of sweet clover, lupine (*Lupinus* sp.; common varieties from Germany), oak (*Quercus* sp.), tomato (*Lycopersicon esculentum*), and sweet potato (*Ipomoea batatas*). The growth and production of fruiting bodies varied greatly on the different media. On oak and cotton there was but a sparse growth, although a few pycnidia were formed. On tomato there was practically no growth. Numerous fruiting bodies were produced on stems of sweet clover (mature and immature), sweet potato, and a fair growth of hyphæ with production of pycnidia on lupine. Mycelium is so sparingly formed that when produced abundantly it is a sign that the medium is not suited to the best development of the fungus. The production of pycnidia, on the other hand, is evidence that the medium is a most favorable one.

abundance of pycnospores are produced on corn meal and rice. On beef agar and bouillon, on the other hand, the pycnidia are comparatively few and mostly sterile. The development of pycnidia on steamed sweet-potato stems was very similar to that found in nature, except that the beaks were longer. A fair growth only was made on string beans and Irish-potato and sweet-potato cylinders. Corn meal and rice, however, are the only media tried on which the growth could be regarded as showing typical development, it being quite apparent in most other cases that the conditions were quite abnormal.

GERMINATION OF PYCNOSPORES

Germination begins in about 6½ to 7 hours in hanging drop cultures. Growth in sterile or hydrant water is slow at first, the germ tube reaching only about one-half to one and one-half times the length of the spore in 24 hours at room temperature (21.5° to 22.5° C.). In nutrient media a much better growth is made. At the end of 48 hours the germ tube reaches a length several times that of the spore and begins branchings. (Pl. XXV, *H*.) Growth is fairly rapid thereafter, although if compared with certain Fusaria it would be regarded as a slow-growing organism both in artificial culture and on the host. Preliminary to germination, the pycnospores swell up, lose their original shape, and become more nearly spherical.

INFLUENCE OF TEMPERATURE ON THE GERMINATION OF PYCNOSPORES

The minimum, optimum, and maximum temperatures for germination of the pycnospores were determined by inoculating about 2 c. c. of rice water in test tubes with spores of the fungus from a young culture on corn meal. One set of cultures was placed in each of six thermostats, the range of temperature being indicated in Table II. Another set was similarly inoculated and placed in the laboratory room as a check. The cultures were examined at the end of 18 hours, and those that had germinated freely were thrown out. The other cultures were continued for 24, 42, or 48 hours, as necessity required.

TABLE II.—*Limiting temperature for the germination of pycnospores.*

Thermostat.	Temperature.	Time in hours.			
		18	24	42	48
	°C.				
VI	11.5 to 12.0	No germination		Slight germination	
VIII	15.0 to 17.0	Slight germination		Fair germination	
Room	21.0 to 22.0	Fair germination			
X	25.0 to 26.0	Good germination			
XII	35.5 to 36.0	do			
XIII	37.4 to 37.5	Fair germination	Fair germination		
XIV	40.0 to 40.5				No germination.

While no absolute limits have been established, Table II shows that only a very slight germination of spores took place in thermostat VI (11.5 to 12° C.) at the end of 24 hours. As the temperature was increased, germination became better until the optimum was reached in thermostats X (25.0° to 26.0° C.) and XII (35.5° to 36.0° C.). Germination was somewhat reduced in thermostat XIII (37.4° to 37.5° C.) and completely prohibited in thermostat XIV (40.0° to 40.5° C.). Of the temperatures tried the minimum for germination would be found in thermostat VI (11.5° to 12.0° C.), the optimum in thermostats X (25.0° to 26.0° C.) and XII (35.5° to 36.0° C.), and the maximum in thermostat XIV (40.0° to 40.5° C.).

VIABILITY OF PYCNOSPORES

Just how long the spores will retain their viability in a dried condition is not known. The pycnospores on material collected on August 22, 1912, and kept in an envelope in the laboratory would not germinate in plates of beef agar made on November 27. Hanging-drop cultures were made with hydrant water in Van Tieghem cells from the same material on December 11, with similarly negative results. On the other hand, pure cultures made on August 15, 1912, on corn meal retained their viability to June 18, 1913. These results are not directly comparable, since there is always a certain amount of moisture present in the medium when the culture is started. Furthermore, as was previously pointed out, this organism produces a considerable amount of liquid on corn meal, even though there is no surplus water present in the culture when inoculated.

INFLUENCE OF TEMPERATURE ON GROWTH

The influence of temperature on the growth of *Plenodomus destruens* in cultures was determined by the use of 10 thermostats ranging in average temperatures from 1.09° to 37.3° C., and in the laboratory with an average temperature of 21.9° C. These temperatures varied somewhat, as will be seen by referring to Table III, where the average maximum and minimum temperature for each thermostat is recorded.

Cultures were made on January 15, 1913, on sterilized rice (1085) in test tubes, it having been previously ascertained that this was a favorable medium for the growth of the fungus. Five tubes were placed in each of the 10 thermostats and one set in the culture room in the laboratory as a check. The cultures were kept in the incubators and under observation for 21 days. Table III contains a record of the growth of the organism in each thermostat and in the laboratory room on the different dates covered by the experiment.

TABLE III.—*Record of growth in laboratory room and in 10 thermostats maintained at different temperatures (°C.).*[1]

Date.	I. Average, 1.09°; maximum, 1.60°; minimum, 0.6°.	II. Average, 4.5°; maximum, 5.7°; minimum, 3.2°.	III. Average, 7.0°; maximum, 9.0°; minimum, 5.4°.	V. Average, 9.0°; maximum, 10.0°; minimum, 8.0°.	VI. Average, 12.6°; maximum, 14.0°; minimum, 11.2°.	VII. Average, 15.2°; maximum, 16.7°; minimum, 13.9°.	IX. Average, 16.4°; maximum, 18.5°; minimum, 15.3°.	X. Average, 17.6°; maximum, 19.5°; minimum, 16.3°.	Room.[2] Average, 21.9°; maximum, 24.5°; minimum, 17.2°.	XI. Average, 30.2°; maximum, 30.8°; minimum, 29.4°.	XII. Average, 37.3°; maximum, 38.0°; minimum, 36.4°.
Jan. 20	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	Sparse hyphal growth.	Some mycelial growth; a few scattered pycnidia.	Very slight growth.
Jan. 21	do.	do.	do.	do.	do.	do.	do.	do.	Increased hyphal growth. Some pycnidia formed.	Increase in hyphal growth and number of pycnidia.	No change.
Jan. 23	do.	do.	do.	do.	do.	do.	do.	do.	Continued good growth and increase in number of pycnidia.	Increase in hyphal growth and number of pycnidia. Yellow discoloration of medium.	Do.
Jan. 24	do.	do.	do.	do.	do.	do.	Scant growth of hyphæ; very few pycnidia.	Scant growth of hyphæ; very few pycnidia.	Pycnidia numerous.	Abundant hyphal growth. Pycnidia fewer than at room temperature.	Do.
Jan. 27	do.	do.	do.	do.	do.	Few pycnidia forming.	Pycnidia fewer than in thermostat X.	Pycnidia many.	Pycnidia numerous; spores exuding in small droplets.	Pycnidia numerous; spores exuding in small droplets; medium yellowed.	Do.
Jan. 30	do.	do.	do.	do.	do.	Pycnidia few.	Pycnidia many; spores oozing but slightly.	Pycnidia many; spores exuding slightly.	do.	Hyphal growth better than at any other temperature.	Do.
Feb. 8	do.	do.	do.	do.	do.	Pycnidia few; slight discharge of spores.	Hyphal growth less than in thermostat X; spores exuding.	Fair hyphal growth; spores exuding.	Best growth, formation of pycnidia, and discharge of spores at this temperature.	Spore discharge yellowish.	Do.
Feb. 6	do.	do.	do.	do.	Sparse growth of hyphæ; a few pycnidia.	A fair growth of hyphæ; pycnidia fairly abundant; spores exuding.	Pycnidia very abundant; spores exuding.	Pycnidia very abundant; spores exuding.	Pycnidia very numerous; spores discharged more abundantly than at any other temperature.	Spores atypical; no further discharge of conidia.	Do.

[1] Temperature readings were made about 9.15 a. m. and 4.15 p. m. each day.

[2] The cultures were kept in a culture room in the middle of the laboratory.

It is seen from Table III and also from figure 2 that the temperatures of thermostats I, II, III, and V (1.09° to 9.0° C.) are prohibitive of growth. A sparse growth took place in thermostat VI (11.2° to 14.0° C.) and reached its maximum growth in the laboratory room (17.2° to 24.5° C.). The best growth was obtained at an average temperature of 21.9° C. and the next best in thermostat XI (29.4° to 30.8° C.). The growth of mycelia in thermostat XI (29.4° to 30.8° C.) was better at the outset than at any other temperature, although the production of pycnidia and spores was not as good at the end of the experiment as in cultures growing in the laboratory room. The medium in thermostat XI was decidedly discolored, a change which did not occur at any other temperature. In

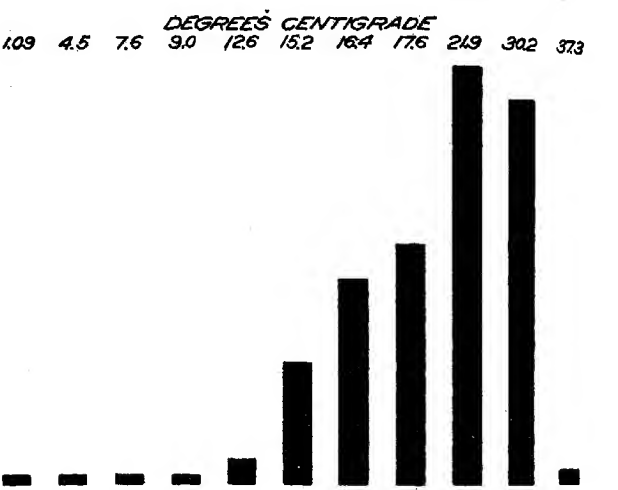

FIG. 1.—Graphic representation of growth on rice at different temperatures.

thermostat XII (36.4° to 38.0° C.) a very slight growth of hyphæ took place during the first three or four days. No further development took place thereafter. While these results do not definitely fix the limiting temperature for growth, they show that the optimum probably lies somewhere between 21.9° and 30.2° C., the minimum close to 12.6° C., and the maximum at about 37.3° C.

At the conclusion of the incubation period all the cultures were taken from the thermostats and placed on a table in the laboratory room. At the end of 10 days there was a good growth in all the tubes except in those that were in thermostat XII (36.4° to 38.0° C.), cultures of which had been killed in 21 days.

A comparison of these results with similar experiments carried on with the pycnidial stage of *Diaporthe batatatis*[1] shows that *Plenodomus destruens* is limited to a narrower range of temperatures in its growth in artificial cultures. The optimum temperature for growth of the dry-rot organism was 3° C. higher than that of the foot-rot fungus. At the lower temperatures the former made as good a growth at an average temperature of 7.5° C. as the latter did at an average temperature of 12.6° C. At the higher temperatures the foot-rot fungus was killed when exposed for 21 days at an average temperature of 37.3° C., while the dry-rot organism made some growth when exposed for 18 days at an average temperature of 37.8° C.

INFLUENCE OF LIGHT ON THE GROWTH AND PRODUCTION OF PYCNIDIA

It was found that the conidial stage of the dry-rot fungus. (*Diaporthe batatatis*) produced pycnidia only sparingly unless exposed to light.[1] Contrary to these results, the foot-rot organism on rice cultures grew equally well in darkness and in the light. Pycnidia were formed in about 3 days, and the spores began exuding in small droplets in about 10 days.

DISSEMINATION OF THE DISEASE

From what we already know of the foot-rot disease it is evident that there are several ways in which the organism may be carried from one place to another. In view of the fact that the pycnospores will live through the winter on the dead vines until as late as May 20, the plants in near-by hotbeds and even in the fields are liable to infection from this source. It has been shown that the organism causes a serious disease of the stem of the plant and grows from there to the roots, forming pycnidia on the surface. It is evident, therefore, that the use of such potatoes for seed might account for a large part of the infections.

There is no way of determining to what extent insects, the wind, and such agencies are responsible for the distribution of the disease, but they, do doubt, play an important part. It is believed that this disease, like many others, is also distributed from one field to another on farm implements, the hoofs of animals, or by means of stable manure, etc. It is a well-known fact that many farmers are careless about the disposition of diseased and decayed sweet potatoes. Without suspecting the risk they are taking, they often throw them on the manure pile or feed them to stock without cooking. In either case the organism, if present on the potatoes, might eventually be carried to the field. The wider distribution of the disease—i. e., from one locality to another—must largely be accounted for by the exchange of seed potatoes and seed plants.

[1] Harter, L. L., and Field, Ethel C. A dry rot of sweet potatoes caused by *Diaporthe batatatis*. U. S. Dept. Agr., Bur. Plant Indus., Bul. 281, 38 p., 4 pl., 1913.

POSSIBLE METHODS OF CONTROL

The suggestions here given for the control of foot-rot are not based on experimental evidence, but on what would seem obvious from a knowledge of the disease and the methods of handling the crop. It has already been pointed out (1) that the disease occurs both on the stem and roots of sweet-potato plants; and (2) that the pycnospores of the fungus can live through the winter and late enough the following spring to infect the new crop. With these facts in mind it will be clear that precautionary and sanitary measures should be employed. One of these should consist in the careful selection of healthy potatoes for seed. Selection should be made preferably in the fall at digging time and any suspicious potatoes should be discarded. They should be carefully examined again in the spring when the disease is more easily recognized, and all those that show any sign of disease should be rejected. While disinfection of the seed in a solution of mercuric chlorid (1:1,000) will not destroy the fungus buried beneath the surface of the potato, it will kill all adhering spores and clean the potatoes so that diseased spots can be more readily detected. After immersing for five minutes in the solution, the potatoes should be rinsed in water and thoroughly dried. It is advisable that disinfection be done on a clear, warm day, just before the potatoes are put in the bed.

Soil that is likely to be infected with the disease should not be used in the preparation of the hotbed. If, however, disease-free soil can not be obtained, then it should be disinfected by steaming for one hour at a temperature of 100° C. If steam sterilization is not feasible, the soil may be soaked in a formaldehyde (40 per cent) solution (1:200). If the latter method of disinfection is employed, the soil should be treated at least 10 days before it is to be used, and it should be occasionally stirred to assist in the escape of the gas.

All decayed, diseased, or discarded potatoes should not be fed raw to stock, or thrown on the manure pile to compost, but should be cooked; neither should the potatoes be thrown on the ground around the hotbed. These practices are too common, and are liable to infect otherwise disease-free beds.

Crop rotation is a good practice, whether for the control of diseases or not, and should be practiced by every farmer. It is not yet known how long this disease retains its vitality in the soil without sweet potatoes as a host, but probably for several years. At least three years should be allowed between crops whenever diseases of this type are found, although it is doubtful if this length of time will completely eradicate it from the soil, but it should reduce it considerably.

SUMMARY

(1) The foot-rot has been hitherto unknown on the sweet potato (*Ipomoea batatas*). It is caused by the fungus *Plenodomus destruens*.

(2) The organism is a very destructive wound parasite of the sweet potato in the vicinity of the Dismal Swamp, Va., and occurs at Cape Charles and Keller, Va.

(3) It kills the plant by the destruction of the cortex of the stem near the ground.

(4) Pycnidia are abundantly formed on the diseased area of the stem about the time the plant dies, or soon thereafter.

(5) The disease, while primarily found on the stem, invades the roots and vines also.

(6) The fungus is cultivable on most artificial media, but gives the highest development on corn meal, rice, and stems of the sweet potato.

(7) The parasitism of the organism has been proved by numerous inoculations of plants grown on the Potomac Flats and in the greenhouse.

(8) Successful infection experiments were carried out with reisolations of the fungus from inoculated plants.

(9) The organism is parasitic on *Ipomoea coccinea*, but not on *I. purpurea* and *I. hederacea*.

(10) Sweet potatoes from storage are decayed by the fungus when inoculated under sterile conditions and kept moist in light.

(11) Light has no apparent effect on the production of fruiting bodies in pure cultures of rice.

(12) The fungus makes its best growth, as measured by abundance and rapidity of sporulation, in rice cultures at an average temperature of about 21.9° C.

(13) The fungus can live through the winter on dead vines of the sweet potato.

(14) The disease is probably disseminated principally by means of "seed roots" and the slips produced therefrom.

(15) Seed beds should be sterilized, and potatoes to be used for seed should be carefully selected.

DESCRIPTION OF PLATES

PLATE XXIII. Parts of sweet-potato plants, showing the presence of pycnidia: *A*, On the stem just above the ground; *B*, on the root.

XXIV. Portion of sweet-potato vines several feet from the hill, showing the results of a natural infection of the foot-rot fungus. The organism was recovered from these vines before being photographed.

XXV. Microscopic characters of the foot-rot fungus: *A*. Section through a pycnidium on the root; *B*, section through a pycnidium on the stem; *C*, hyphae, from artificial culture; *D* and *E*, chlamydospore-like bodies found on the host and in some culture media; *F*, pycnospores; *G*, club-shaped bodies often found in pycnidia; *H*, germinating pycnospores.

XXVI. Two sweet-potato plants in pots, demonstrating the parasitism of the foot-rot fungus: *A*, Inoculated; *B*, not inoculated.

XXVII. Nine-day-old cultures on synthetic agar: *A*, The conidial stage of *Diaporthe batatatis; B*, *Plenodomus destruens.*

XXVIII. Sweet potatoes inoculated with *Plenodomus destruens: A*, Inoculated at the end; *B*, a section of *A* showing extent of rot; *C*, inoculated at the side; *D*, section of *C* showing the extent of rot.

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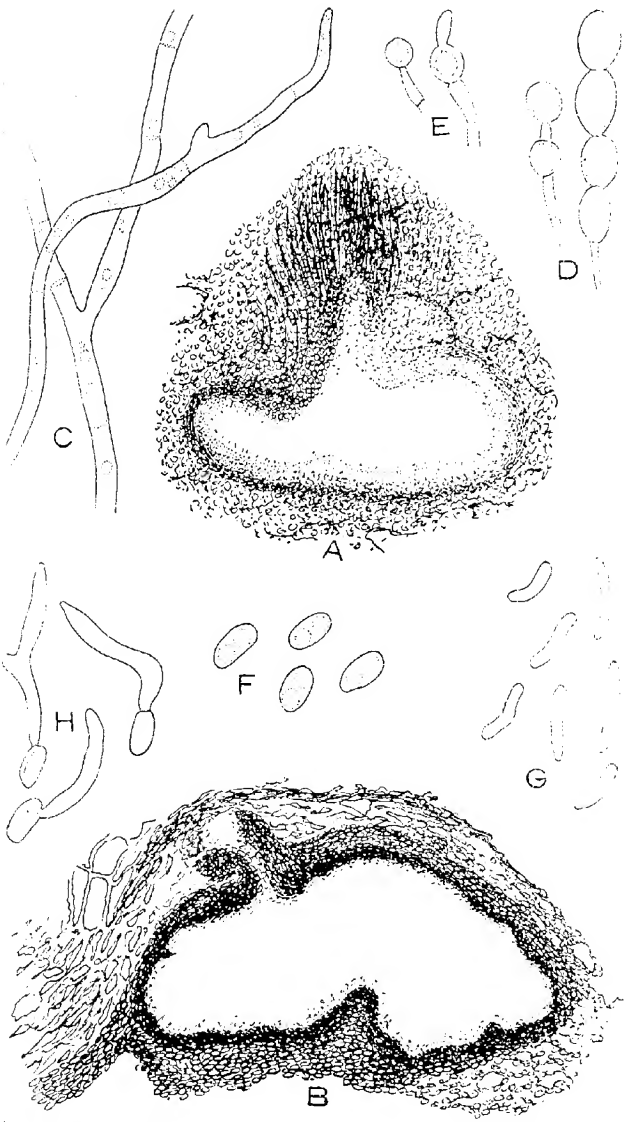
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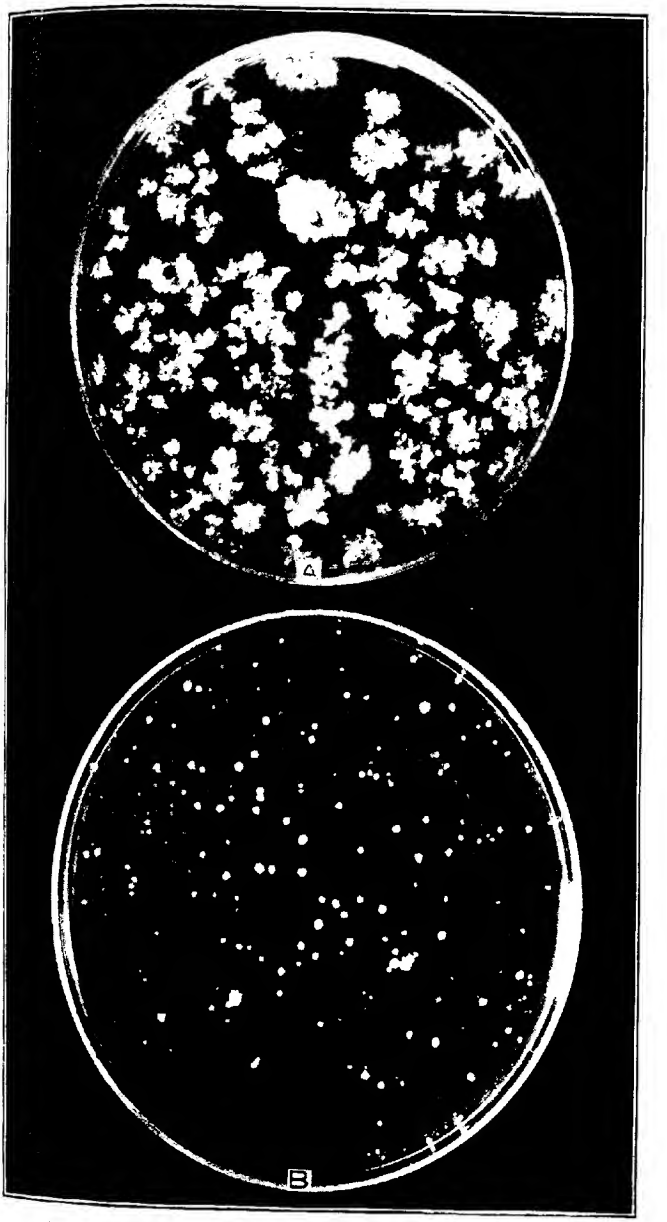
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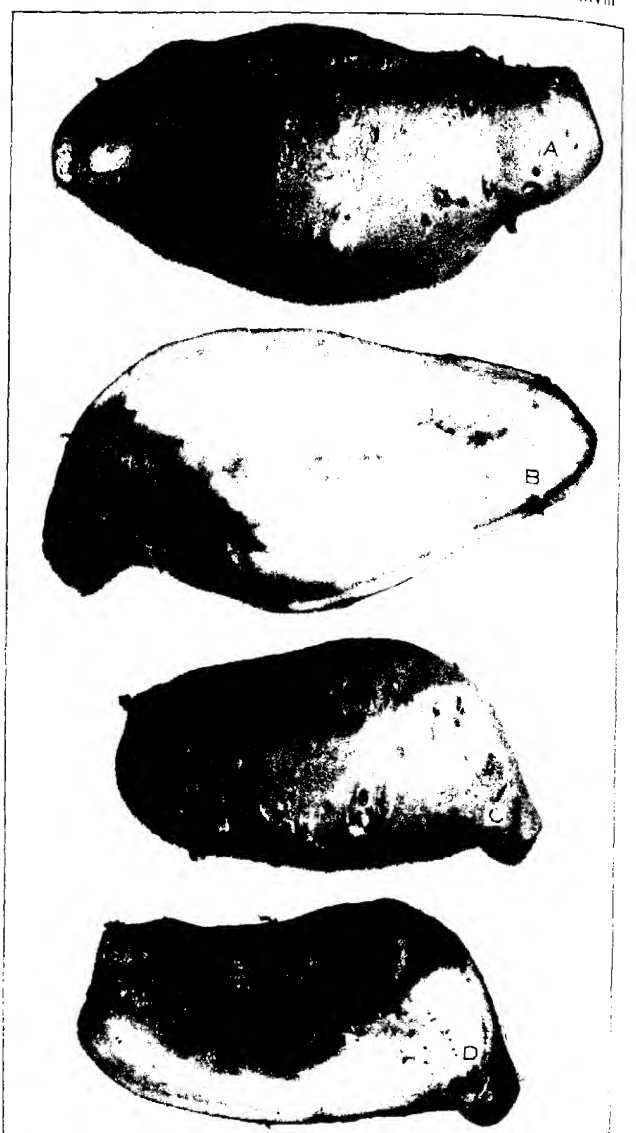
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ENVIRONMENTAL INFLUENCES ON THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF WHEAT

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INTRODUCTION

A former series of experiments [1] conducted in the Bureau of Chemistry showed that neither the composition nor the physical characteristics of wheat are to any great extent hereditary. The protein, gluten, and ash contents, as well as the size of the berry, the weight of a bushel, and the flintiness of the kernel, were found to be dependent upon the climatic conditions prevailing during the growing period of the plant. Seed of Kansas wheat containing 20 per cent of protein and showing 100 per cent of flinty kernels and seed of California wheat containing 10 per cent of protein with 13 per cent of flinty kernels when grown side by side in South Dakota yielded crops of identical composition and physical appearance. The same was true of these Kansas and California seeds when grown in California. The crops grown in California were, however, entirely unlike those grown in South Dakota, owing to the great difference in climatic conditions. It was shown in a most conclusive manner that environment plays a major part in influencing both the chemical composition and the physical appearance of a wheat crop. Cropping through a number of generations under widely different environments therefore does not alter permanently or make a noticeable impression upon the transmissible physical and chemical properties of wheat.

Similar experiments, involving the transference of soil, are reported by Shaw and Walters.[2] In the main, their observations, based on crops grown throughout a period of three years in one locality, harmonize with the conclusions here presented, which are founded on the wider range of experimental data now at hand, involving crops grown for four years on three different types of soil in three different localities having widely

[1] Le Clerc, J. A., and Leavitt, Sherman. Tri-local experiments on the influence of environment on the composition of wheat. U. S. Dept. Agr., Bur. Chem. Bul. 128, 18 p., 1910.

[2] Shaw, G. W., and Walters, E. H. A progress report upon soil and climatic factors influencing the composition of wheat. Cal. Agr. Exp. Sta. Bul. 216, p. 549-574, 1911.

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varying climatic conditions. In some particulars, however, the conclusions which seemed justifiable from their experiments are not borne out by these more extensive data.

The experiments discussed in this article were designed to study further the environmental influences and to show the rôle exerted by the soil and the part played by climatic conditions, such as rainfall, sunshine, humidity of the atmosphere, temperature, winds, and elevation above sea level. As in the case of the previous experiments,[1] they were carried on in cooperation with the Office of Cereal Investigations of the Bureau of Plant Industry. The agricultural experiment stations of Maryland, Kansas, and California cooperated by growing the crops.

CONDUCT OF THE EXPERIMENTS

In order to distinguish between the rôle played by soil and that by environment other than soil, samples of soil were interchanged among three localities, Maryland (College Park), Kansas (Hays), and California (Davis), which differ widely in climatic conditions. From each locality sections of a normally fertile wheat-producing soil 5 feet square and 3 feet deep were dug up in 3-inch layers, sacked, and replaced in the same original position. To obviate any differences due to this manipulation a portion of soil 5 feet square and 3 feet deep from each locality was likewise dug up in 3-inch layers, sacked, and stored until the soils from the two other localities had arrived, when all three samples were placed in their respective positions. A fourth plat of soil of the same size was allowed to remain undisturbed in each locality to determine whether the treatment to which the three other soils had been subjected would exert any influence on the composition of the grain. Thus, there were 12 experimental plats, 4 in each locality, as shown in the following plan:

TWELVE EXPERIMENTAL PLATS

California:
- Plat of undisturbed California soil, or check plat.
- Plat of disturbed California soil.
- Plat of Kansas soil.
- Plat of Maryland soil.

(Each taken up in 3-inch layers and replaced in original order.)

Kansas:
- Plat of undisturbed Kansas soil, or check plat.
- Plat of disturbed Kansas soil.
- Plat of California soil.
- Plat of Maryland soil.

(Each taken up in 3-inch layers and replaced in original order.)

Maryland:
- Plat of undisturbed Maryland soil, or check plat.
- Plat of disturbed Maryland soil.
- Plat of California soil.
- Plat of Kansas soil.

(Each taken up in 3-inch layers and replaced in original order.)

[1] Le Clerc and Leavitt. Op. cit.

During the first two years, 1908 and 1909, Crimean wheat obtained from seed grown in Kansas was used on all 12 plats. As this variety was not adapted to conditions prevailing in Maryland and California, Turkey wheat was selected for 1910, 1911, and 1912. The change from Crimean to Turkey wheat did not interfere, however, with the object of the experiment, which was to determine the influence exerted by climatic conditions and soil on the composition of the crop.

The following determinations were made according to the methods given in Bulletin 107, Revised, of the Bureau of Chemistry, entitled "Official and Provisional Methods of Analysis."

Water; weight of 1,000 grains; weight of a bushel; flinty grains; nitrogen; alcohol-soluble nitrogen; fat; fiber; pentosans; sugars; ash; phosphoric acid; and potash. The alcohol-soluble nitrogen was determined by treating a certain quantity of ground wheat with a 70 per cent solution of alcohol at ordinary temperature, with frequent shaking, for several hours, and then allowing the solution to stand overnight. An aliquot part was taken and the nitrogen therein determined. The amount of nitrogen thus obtained divided by the total quantity of nitrogen in the sample gave the gliadin number.

TABULATION OF DATA

The data are collected in a number of tables. In Table I, first column, is given the analysis of the original seed grown in Kansas in 1908, which was used as seed on all the plats for the following year's crop. The other analyses in Table I and the data in Tables II to IV were obtained on crops grown in 1909, 1910, 1911, and 1912, the results being grouped by locality. The data from the different soil plats and the check-soil plat in each locality are arranged in adjacent columns in Table I. In Table II the same data, exclusive of check-plat data, are rearranged, the results from the same soils being grouped in adjacent columns. Averages derived from these data are given in Tables III, IV, and V. In Table III are shown the averages of all the constituents from crops grown in California, Kansas, and Maryland, not including the check-soil plat, throughout the four years of the experiment. Table IV gives the averages obtained from data on the crops grown on the soils of California, Kansas, and Maryland for each of the three localities and for all four years. Finally, in Table V are shown the averages for the undisturbed or check-soil plats and for the corresponding plats in which the soil had been taken up in 3-inch layers and replaced.